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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	10/086,208
Filing Date	February 28, 2002
First Named Inventor	Goldstein
Art Unit	1648
Examiner Name	J. Parkin
Attorney Docket Number	GGP2CUSA

Total Number of Pages in This Submission: 39

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
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- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC (**Appeal Notice, Brief, Reply Brief**)
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

3 pp. Request for Certificate of Correction under 35 USC Section 254

3 pp. Forms PTO/SB/44

Remarks

18. Copy of specification pages with words or phrases marked and highlighted in blue

14 pp. Copy of original issued Patent pages with errors marked in red

Customer No. 00270

Certificate
JUN 08 2006
of Correction

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	HOWSON AND HOWSON
Signature	Mary E. Bak
Printed name	Mary E. Bak
Date	June 2, 2006 — Reg. No. 31,215

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature	Lynn Brown-Fischer
Typed or printed name	Lynn Brown-Fischer — Date: June 2, 2006

JUN 9 2006

GGP2CUSA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No.	: 10/086,208	Confirmation No.: 3296
Applicant	: Goldstein	
Filed	: February 28, 2002	
Patent No.	: 7,008,622	
Issued	: March 7, 2006	
TC/A.U.	: 1648	
Examiner	: J. Parkin	
Customer No.	: 00270	
Title	: METHODS AND COMPOSITONS FOR IMPAIRING MULTIPLICATION OF HIV-1	

ATTN: Certificate of Corrections Branch
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 35 USC SECTION 254

The following errors were found in the above-identified patent:

(1) First page of patent, (60) Related U.S. Application Data, replace "5,871,994" with -- 5,891,994 -- ;

(2) Col. 1, line 48, replace "Coomnbs" with -- Coombs -- ;

Certificate of Mailing Under 37 CFR 1.8
I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope Addressed to: Certificate of Corrrections Branch, Commissioner for Patents, Box 1450, Alexandria, VA 22313-1450 on June 2, 2006.

Signature: Lynn Brown-Fischer Printed: Lynn Brown-Fischer

(3) Col. 2, line 13, replace "JAAM" with -- JAMA -- ;

(4) Col. 2, line 66, replace "polygonal" with -- polyclonal -- ;

(5) Col. 3, line 49, replace "areproduced" with -- are produced -- ;

(6) Col. 3, line 59, replace "lone" with -- one -- ;

(7) Col. 4, line 3, replace "termninal" with -- terminal -- ;

(8) Col. 4, line 25, replace "Gin" with -- Gln -- ;

(9) Col. 4, line 28, replace "Gin" with -- Gln -- ;

(10) Col. 4, line 35, replace "Epitope ID" with -- Epitope III -- ;

(11) Col. 5, line 11, replace "Epitope It" with -- Epitope II -- ;

(12) Col. 9, line 11, Table 1, replace "45,000 (65)" with – 45,000 (55) -- ;

(13) Col. 10, line 64, replace "NLAID" with -- NIAID -- ;

(14) Col. 11, line 48, replace "Gin" with -- Gln -- ;

(15) Col. 12, line 56, replace "SEQ if)" with -- SEQ ID -- ;

(16) Col. 13, line 66, replace "<u>Lys-Glu-Leu-Gly-Ile-Ser-Tyr-Gly-Arg-Lys-Lys-amide</u>" with -- <u>Lys-Glu-Leu-Gly-Ile-Ser-Tyr-Gly-Arg-Lys-Lys</u>-amide -- ;

(17) Col. 16, line 2, replace "polypeptidelpeptide" with -- polypeptide/peptide -- ;

(18) Col. 16, line 22, replace "11478-11492" with -- 11478-11482 -- ;

(19) Col. 18, line 37, replace "I,IV" with -- III, IV -- ;

(20) Col. 19, Line 34, replace "polyrations," with -- polycations -- ;

(21) Col. 19, line 60, replace "990%," with -- 90%, -- ;

(22) Col. 29, line 44, Table 8, delete "Pro-Gly-Ser-"

(23) Col. 36, lines 35-36, replace "cross-presented in 61% of HIV-1 strains" with --cross-reactivity with most of these variants, represented in 61% of HIV-1 stains -- ;

(24) Col. 40, line 63, replace "ME75" with -- MF75 -- ;

(25) Col. 44, line 34, replace "calori-" with -- colori- -- ;

It is requested that a Certificate of Correction be issued to correct the above error in accordance with the enclosed Form PTO 1050, which is submitted herewith.

These errors are typographical in nature and make no substantive changes. All errors were made by the USPTO, therefore, no fee is due for correction of these errors.

Enclosed for each correction is a photocopy of the original specification page with the relevant words or phrases highlighted in blue and the corresponding original patent with errors marked in red. These documents will support the USPTO errors.

The Director of the US Patent and Trademark Office is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing, or during prosecution of this application to Deposit Account No. 08-3040.

Respectfully submitted,
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PTO/SB/44 (04-05)
Approved for use through 04/30/2007. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO : 7,008,622

APPLICATION NO. : 10/086,208

ISSUE DATE : March 7, 2006

INVENTOR(S) : Goldstein

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

(1) First page of patent, (60) Related U.S. Application Data, replace "5,871,994" with 5,891,994 -- ;

(2) Col. 1, line 48, replace "Coomnbs" with -- Coombs -- ;

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(11) Col. 5, line 11, replace "Epitope It" with -- Epitope II -- ;

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(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

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APPLICATION NO. : 10/086,208

ISSUE DATE : March 7, 2006

INVENTOR(S) : Goldstein

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(14) Col. 11, line 48, replace "Gin" with -- Gln -- ;

(15) Col. 12, line 56, replace "SEQ if)" with -- SEQ ID -- ;

(16) Col. 13, line 66, replace "Lys-Glu-Leu-Gly-Ile-Ser-Tyr-Gly-Arg-Lys-Lys-amide" with -- Lys-Glu-Leu-Gly-Ile-Ser-Tyr-Gly-Arg-Lys-Lys-amide -- ;

(17) Col. 16, line 2, replace "polypeptidelpeptide" with -- polypeptide/peptide -- ;

(18) Col. 16, line 22, replace "11478-11492" with -- 11478-11482 -- ;

(19) Col. 18, line 37, replace "I,IV" with -- III, IV -- ;

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(21) Col. 19, line 60, replace "990%," with -- 90%, -- ;

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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO : 7,008,622

APPLICATION NO. : 10/086,208

ISSUE DATE : March 7, 2006

INVENTOR(S) : Goldstein

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

(22) Col. 29, line 44, Table 8, delete "Pro-Gly-Ser-"

(23) Col. 36, lines 35-36, replace "cross-presented in 61% of HIV-1 strains" with --cross-reactivity with most of these variants, represented in 61% of HIV-1 stains -

(24) Col. 40, line 63, replace "ME75" with -- MF75 -- ;

(25) Col. 44, line 34, replace "calori-" with -- colori- -- ;

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ERROR (1)

US007008622B2

(12) **United States Patent**
Goldstein

(10) **Patent No.: US 7,008,622 B2**
(45) **Date of Patent: Mar. 7, 2006**

(54) **METHODS AND COMPOSITIONS FOR IMPAIRING MULTIPLICATION OF HIV-1**

(75) Inventor: **Gideon Goldstein**, Short Hills, NJ (US)

(73) Assignee: **Thymon, L.L.C.**, Short Hills, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 332 days.

(21) Appl. No.: **10/086,208**

(22) Filed: **Feb. 28, 2002**

(65) **Prior Publication Data**

US 2003/0194408 A1 Oct. 16, 2003

Related U.S. Application Data

(60) Division of application No. 09/451,067, filed on Nov. 30, 1999, now Pat. No. 6,525,179, which is a division of application No. 09/113,921, filed on Jul. 10, 1998, now Pat. No. 6,193,981, which is a continuation-in-part of application No. 08/893,853, filed on Jul. 11, 1997, now Pat. No. 5,871,994.

(51) **Int. Cl.**
A61K 39/42 (2006.01)

(52) **U.S. Cl.** **424/139.1**; 424/148.1; 424/160.1; 424/188.1; 424/208.1; 530/387.9; 530/388.35; 530/389.4

(58) **Field of Classification Search** 530/388.35, 530/389.4; 424/188.1, 208.1, 190.1, 160.1

See application file for complete search history.

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(Continued)

Primary Examiner—James Housel
Assistant Examiner—Jeffrey S. Parkin
(74) *Attorney, Agent, or Firm*—Howson and Howson

(57) **ABSTRACT**

A composition which elicits antibodies to greater than 95%, and even greater than 99%, of the known variants of HIV-1 Tat protein contains at least one peptide or polypeptide of the formula of Epitope I (based on amino acids 2–10 of HIV-1 Tat consensus sequence) and optionally one or more of a peptide or polypeptide of Epitope II (based on amino acids 41 to 51 of that sequence), of Epitope III (based on amino acids 52–62 of that sequence), or of Epitope IV (based on amino acids 62 through 72 of that sequence with a C-terminal Pro). Vaccinal and pharmaceutical compositions can contain the antibodies induced by the peptide compositions for use in passive therapy. Diagnostic compositions and uses are described for assessing the immune status of vaccinated patients.

10 Claims, 4 Drawing Sheets

ERRORS(2), (3), (4)

METHODS AND COMPOSITIONS FOR IMPAIRING MULTIPLICATION OF HIV-1

CROSS-REFERENCE TO OTHER INVENTIONS

This is a divisional of U.S. patent application Ser. No. 09/451,067, filed Nov. 30, 1999 now U.S. Pat. No. 6,525,179, which is a divisional of U.S. patent application Ser. No. 09/113,921, filed Jul. 10, 1998, now U.S. Pat. No. 6,193,981, issued Feb. 27, 2001, which is a continuation-in-part of U.S. patent application Ser. No. 08/893,853, filed Jul. 11, 1997, now U.S. Pat. No. 5,891,994, issued Apr. 6, 1999.

BACKGROUND OF THE INVENTION

The present invention relates generally to compositions and methods useful for inhibiting the multiplication of human immunodeficiency virus-1 (HIV-1) in infected patients, symptomatic or asymptomatic, and for attenuating HIV-1 multiplication during primary infection in previously uninfected subjects, thus minimizing progression to AIDS.

High plasma levels of human immunodeficiency virus type 1 (HIV-1) RNA are found during primary infection with HIV-1, the seroconversion illness, (C. Baumberger et al, AIDS, 7:(suppl 2): S59 (1993); M. S. Saag et al, Nature Med., 2:625 (1996)), after which they subside as the immune response controls the infection to a variable extent. Post seroconversion, lower but detectable levels of plasma HIV-1 RNA are present, and these levels rise with disease progression to again attain high levels at the AIDS stage (M. S. Saag et al, Nature Med., 2:265 (1996)). Approximately 50% of subjects have a symptomatic illness at seroconversion (B. Tindall and D. A. Cooper, AIDS, 5:1 (1991)) and symptomatic seroconversion is associated with an increased risk for the development of AIDS, probably because a severe primary illness is likely related to an early and extensive spread of HIV.

Inhibition of viral multiplication during the initial infection will likely reduce the subsequent development of chronic viremia leading to AIDS. Current medical practice, with administration of antiviral drugs for defined "at risk" situations, such as needle sticks with contaminated blood or pregnancy in HIV infected mothers, supports this concept.

Post seroconversion levels of HIV-1 RNA in plasma have proven to be the most powerful prognosticator of the likelihood of progression to AIDS (J. W. Mellors et al, Science, 272:1167 (1996); M. S. Saag et al, Nature Med., 2:265 (1996), R. W. Coomnbs et al, J. Inf. Dis., 174:704 (1996); S. L. Welles et al, J. Inf. Dis., 174:696 (1990)). Other measures of viral load, such as cellular RNA (K. Saksela et al, Proc. Natl. Acad. Sci. USA, 91:1104 (1994)) and cellular HIV proviral DNA (T-H. Lee et al, J. Acq. Imm. Def Syndromes, 7:381 (1994)) similarly establish the importance of the initial infection in establishing viral loads that determine future disease progression.

Thus, any intervention that inhibits HIV-1 infectivity during initial infection and/or lowers viral load post seroconversion is likely to have a favorable influence on the eventual outcome, delaying or preventing progression to AIDS.

A variety of methods are now employed to treat patients infected with human immunodeficiency virus (HIV-1), including treatment with certain combinations of protease inhibitor drugs. Unfortunately, however, this type of treatment is associated with serious side effects in some patients. Alternatively, vaccines are under development for control of the spread of HIV-1 to uninfected humans. However, this

effort has largely been directed to proteins of the virus, expressed on the surface of infected cells, which are recognized by cytotoxic T cells with elimination of the infected cells, while free virus is blocked and cleared by antibody to surface antigens of the virion. Limitations of this mode of vaccination are readily apparent for HIV-1, which has demonstrated a great diversity in immunogenic viral epitopes and rapid mutational variations that occur within and between individuals (B. D. Preston et al., *Science*, 242:1168 (1988); J. D. Roberts et al., *Science*, 242:1171 (1988); A. R. Meyerhans et al., *Cell*, 58:901 (1989); K. Kusumi et al., *J. Virol.*, 66:875 (1992), B. A. Larder et al., *Science*, 243:1731 (1989); M. S. Sang et al., *N. Engl. J. Med.*, 329:1065 (1993); M. A. Sande, et al., *JAAM*, 270:2583 (1993); M. Seligmann et al., *Lancet*, 343:871 (1994); G. Meyers et al., *Human retroviruses and AIDS* 1993, *I-V. A compilation and analysis of nucleic acid and amino acid sequences.*. Los Alamos National Laboratory, Los Alamos, N.Mex.)

Variation in strains of HIV-1 and frequent mutations of virion proteins have prevented successful application of conventional vaccine approaches (W. E. Paul, *Cell*, 82:177 (1995); J. E. Osborn, *J. Acq. Imm. Def. Syndr. Hum. Retrovirol.*, 9:26 (1995)). Mutation and selection of resistant variants is the central problem in developing a successful HIV-1 vaccine (M. D. Daniel et al., *Science*, 258:1938 (1992); N. L. Letvin, *N. Engl. J. Med*, 329:1400 (1993); M. Clerici et al., *AIDS*, 8:1391 (1994); S. M. Wolinsky et al, Science, 272:537 (1996)).

Other approaches to HIV-1 treatment have focused on the transactivating (tat) gene of HIV-1, which produces a protein (Tat) essential for transcription of the virus. The tat gene and its protein have been sequenced and examined for involvement in proposed treatments of HIV (see, e.g., U.S. Pat. No. 5,158,877, U.S. Pat. No. 5,238,882; U.S. Pat. No. 5,110,802; International Patent Publication No. WO92/07871, published May 14, 1992; International Patent Publication No. WO91/10453, published Jul. 25, 1991, International Patent Publication No. WO91/09958, published Jul. 11, 1991; International Patent Publication No. WO87/02989, published May 21, 1987). Tat protein is released extracellularly, making it available to be taken up by other infected cells to enhance transcription of HIV-1 in the cells and to be taken up by noninfected cells, altering host cell gene activations and rendering the cells susceptible to infection by the virus. Uptake of Tat by cells is very strong, and has been reported as mediated by a short basic sequence of the protein (S. Fawell et al., Proc. Natl. Acad. Sci., USA, 91:664–668 (1994)).

International Patent Publication No. WO92/14755, published Sep. 3, 1992, relates to the Tat protein and to the integrin cell surface receptor capable of binding to the Tat protein. Two Tat sequences that bind integrin are identified, which are the basic region or domain which is the dominant binding site for the integrin, having a peptide sequence of -Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg- (SEQ ID NO: 4), as well as -Gly-Arg-Gly-Asp-Ser-Pro- (SEQ ID NO: 5). This specification demonstrates that a number of peptides corresponding to these Tat sequences and the corresponding integrins block in vitro cell binding to Tat coated plates, as do antibodies to the appropriate integrins. However, the specification also shows that these reagents do not block uptake of functional Tat by cells (see Example 9 in WO92/14755), thus nullifying the proposed mechanism of action for therapeutic benefit in HIV infection. The Tat sequences described in this international application are distinct from the peptide immunogens of the present invention.

Both monoclonal and polygonal antibodies to Tat protein have been readily produced in animals and shown to block

ERRORS (4), (5), (6), (7), (8), (9), (10)

uptake of Tat protein in vitro (see, e.g., D. Brake et al, J. Virol., 64:962 (1990); D. Mann et al, EMBO J., 10:1733 (1991); J. Abraham et al, cited above; P. Auron et al, cited above; M. Jaye et al, cited above; G. Zauli et al, cited above). More recent reports showed that monoclonal or polyclonal antibodies to Tat protein added to tissue culture medium attenuated HIV-1 infection in vitro (L. Steinaa et al, Arch. Virol., 139:263 (1994); M. Re et al, J. Acq. Imm. Def. Syndr. Hum. Retrovirol., 10:408 (1995); and G. Zauli et al, J. Acq. Imm. Def. Syndr. Hum. Retrovirol., 10:306 (1995)).

The inventor's own publication (G. Goldstein, Nature Med., 2:960 (1996); see also, International Patent Publication No. WO95/31999, published Nov. 30, 1995) reviewed the evidence indicating that secretion of HIV-1 Tat protein from infected cells and uptake by both infected and uninfected cells was important for the infectivity of HIV-1. Previous studies also showed that antibodies to Tat protein in vitro blocked uptake of Tat and inhibited in vitro infectivity. Goldstein proposed active immunization of mammals to induce antibodies to HIV-1 Tat protein as a potential AIDS vaccine.

Despite the growing knowledge about HIV-1 disease progression, there remains a need in the art for the development of compositions and methods for treatment of HIV-1, both prophylactically and therapeutically, which are useful to lower the viral levels of HIV-1 for the treatment and possible prevention of the subsequent, generally fatal, AIDS disease.

SUMMARY OF THE INVENTION

In one aspect, the invention provides as a novel composition comprising a peptide or polypeptide, which comprises an amino acid sequence selected from the formula referred to as Epitope I: R1-Val-Asp-Pro-Y-Leu-Glu-Pro-R2 (SEQ ID NO: 36), wherein Y is variously Arg, Lys, Ser or Asn. The N-terminal R1 may represent hydrogen (i.e., the hydrogen on the unmodified N terminal amino acid), or a lower alkyl, or a lower alkanoyl. R1 may also include a sequence of between 1 to about 5 amino acids, optionally substituted with a lower alkyl or lower alkanoyl. In one embodiment, R1 is -X-Pro-, wherein X is Glu or Asp. Preferably, R1 represents 2 amino acids. The C-terminal R2 can also represent the hydroxyl group on the C terminal amino acid or an amide. To enhance titer R2 is preferably a sequence of between 1 to about 14 additional amino acids amidated at the carboxyl terminus. In a preferred embodiment, R2 is -Trp-Lys-His-Pro-Gly-Ser- amide (SEQ ID NO: 10). The peptides or polypeptides of these compositions areproduced synthetically or recombinantly. This composition may take the form of one or more of the above-described peptides expressed as a synthetic peptide coupled to a carrier, or expressed as a multiple antigenic peptide, or the selected peptides may be expressed within a recombinantly produced protein. This composition is designed to induce antibodies reactive with greater than 95% of the known variants of the HIV-1 Tat protein.

In another aspect, the above-described composition further contains lone or more additional peptide or polypeptide(s) which represent other amino acid sequences which correspond to amino acid residues 2 or 4 to 10 of an HIV-1 Tat protein. These optional amino acid sequences are described in detail below. These sequences are preferably from an HIV-1 strain with a Tat protein variant at that location.

In another aspect, the invention provides a novel composition comprising a peptide or polypeptide of the formula referred to as Epitope II: R3-Lys-X-Leu-Gly-Ile-Ser-Tyr-Gly Arg-Lys-Lys-R4 (SEQ ID NO: 37). According to this formula, X is Gly or Ala. The N termninal R3 may represent hydrogen (i.e., the hydrogen on the unmodified N terminal amino acid), or may be a lower alkyl or a lower alkanoyl. R3 may also include a sequence of between 1 to about 5 amino acids, optionally substituted with a lower alkyl or lower alkanoyl. The C terminal R4 may be the free hydroxyl of the C terminal amino acid, or an amide, or a sequence of one or up to about 5 additional amino acids, optionally substituted with an amide. The peptides or polypeptides of these compositions are produced synthetically or recombinantly, provided that the recombinant Epitope II peptide is situated at the C terminus of the recombinant protein. This composition may take the form of one or more of the above-described peptides expressed as a synthetic peptide coupled to a carrier, or expressed as a multiple antigenic peptide. This composition is designed to induce antibodies reactive with greater than about 95% of the known variants of HIV-1 Tat protein.

In yet a further aspect, this invention provides a composition comprising a peptide or polypeptide of the formula referred to as Epitope III: R5-Arg-Arg-X-Z-A-Y-Ser-R6 (SEQ ID NO: 38), wherein X is selected from the group consisting of Ala, Pro, Ser and Gin;.wherein Y is selected from the group consisting of Asp, Asn, Gly and Ser; wherein Z is selected from the group consisting of Pro and His; and wherein A is selected from the group consisting of Gin and Pro. The N terminal R5 is hydrogen, a lower alkyl, a lower alkanoyl, or a sequence of between 1 to about 3 amino acids, optionally substituted with a lower alkyl or lower alkanoyl. In a preferred embodiment R5 is -Gln-Arg-, optionally modified as above. The C terminal R6 is either a free hydroxyl or an amide. A preferred embodiment of such a composition contains at least three Epitope ID peptides, i.e., -Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser- (amino acids 54–62 of SEQ ID NO: 1), -Gln-Arg-Arg-Arg-Ala-His-Gln-Asp-Ser- (amino acids 2–10 of SEQ ID NO: 65), and -Gln-Arg-Arg-Arg-Ala-Pro-Pro-Asp-Ser- (amino acids 264–272 of SEQ ID NO: 3), optionally modified as above. Other peptides or polypeptides representative of amino acids 56–62 of Tat, but having different sequences from that of the above formula may also be included in the composition. The peptides or polypeptides of these compositions are produced synthetically or recombinantly. This composition may take the form of one or more of the above-described peptides expressed as a synthetic peptide coupled to a carrier, or expressed as a multiple antigenic peptide, or the selected peptides may be expressed within a recombinantly produced protein. This composition is designed to induce antibodies reactive with greater than about 75% of all known variants of HIV-1 Tat protein.

In still a further aspect, this invention provides a composition comprising a peptide or polypeptide of the formula referred to as Epitope IV: R7-Ser-Gln-X-His-Gln-Y-Ser-Leu-Ser-Lys-Gln-Pro-R8 (SEQ ID NO: 39), wherein X is selected from the group consisting of Asn and Thr; and wherein Y is selected from the group consisting of Ala and Val. The N terminal R7 may be hydrogen, a lower alkyl, a lower alkanoyl, or a sequence of between 1 to about 3 amino acids, optionally substituted with a lower alkyl or lower alkanoyl. The C terminal R8 may be a free hydroxyl, an amide, or a sequence of one or up to about 3 additional amino acids, optionally substituted with an amide. A preferred Epitope IV peptide is -Ser-Gln-Thr-His-Gln-Ala-Ser-Leu-Ser-Lys-Gln-Pro- (SEQ ID NO: 40). The peptides or polypeptides of these compositions are produced syntheti-

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cally or recombinantly. This composition may take the form of one or more of the above-described peptides expressed as a synthetic peptide coupled to a carrier, or expressed as a multiple antigenic peptide, or the selected peptides may be expressed within a recombinantly produced protein. This composition is designed to induce antibodies reactive with greater than 64% of all known variants of HIV-1 Tat protein.

In still another aspect, this invention provides composition described above that contains peptides or polypeptides which comprise one or more Epitope I peptides in combination with one or more Epitope II peptides, and/or one or more Epitope III peptides, and/or one or more Epitope IV peptides. Such compositions can combine appropriate Epitope peptides, so as to provide for a composition than induces antibodies reactive with greater than about 99% of all known HIV-1 Tat proteins.

In yet a further aspect, the invention provides a synthetic gene which encodes sequentially a peptide or polypeptide that contains at least one Epitope I amino acid sequence defined above, optionally with a carboxy terminal Epitope II peptide, or contains at least two Epitope I amino acid sequences. The synthetic gene may contain each amino acid sequence separated by a spacer sequence, or may express each peptide/polypeptide in an open reading frame with a carrier protein. The synthetic gene may be separated from the carrier protein by a spacer if the spacer is fused to an Epitope I sequence, leaving an Epitope II sequence at the carboxy terminus of the recombinant protein. Further embodiments include multiple Epitope I peptides fused together and to the carrier protein.

In yet a further aspect, the invention provides a synthetic molecule, e.g., a vector, comprising the above-described synthetic gene, operatively linked to regulatory nucleic acid sequences, which direct and control expression of the product of the synthetic gene in a host cell.

In another aspect, the invention provides a recombinant virus which contains the above described synthetic gene or synthetic molecule, which virus is capable of expressing multiple copies of the product of the gene or molecule in a host cell. The virus is non-pathogenic to humans.

In yet another aspect, the invention provides a commensal bacterium which contains the above described synthetic gene or synthetic molecule, which bacterium is capable of expressing multiple copies of the product of the gene or molecule and inducing antibodies in a mammalian host.

In still a further aspect, the invention provides an isolated antibody composition which is directed against a peptide or polypeptide of the compositions described above. Antibodies may also be obtained against multiple components of the compositions described above. This antibody is produced by immunizing a mammal with a peptide/polypeptide composition of the invention, a synthetic gene or synthetic molecule of the invention; a recombinant virus or commensal bacterium of the invention; and isolating and purifying antibody from said immunized mammal. Alternatively, the antibody may be a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a humanized antibody, a human antibody, or mixtures thereof.

Thus, another aspect of the invention is a pharmaceutical composition useful for inducing antibodies that react with greater than 95%, and preferably greater than 99%, of the known HIV-1 Tat proteins. These induced antibodies can impair the multiplication of HIV-1. The pharmaceutical composition comprises at least one of the recombinant or synthetic peptide/polypeptide compositions described above; the synthetic gene/molecule described above; the recombinant virus described herein, or the commensal bacterium described herein, in a pharmaceutically acceptable carrier.

Still a further aspect of the invention is a pharmaceutical composition useful for impairing the multiplication of HIV-1, this composition containing an above described antibody composition.

In yet a further aspect of the invention, a method for reducing the viral levels of HIV-1 involves exposing a human to antibody-inducing pharmaceutical compositions described above, actively inducing antibodies that react with most HIV-1 Tat proteins, and impairing the multiplication of the virus in vivo. This method is appropriate for an HIV-1 infected subject with a competent immune system, or an uninfected or recently infected subject. The method induces antibodies which react with HIV-1 Tat proteins, which antibodies reduce viral multiplication during any initial acute infection with HIV-1 and minimize chronic viremia which leads to AIDS.

In still another aspect, the invention provides a method for reducing the viral levels of HIV-1 by administering to a human, who is incapable of mounting an effective or rapid immune response to infection with HIV-1, a pharmaceutical composition containing the antibody compositions described above. The method can involve chronically administering the composition.

Yet other aspects of the invention include methods for producing the compositions described above, as well as host cells transfected with such compositions.

Still another aspect of this invention is a kit useful for the measurement and detection of titers and specificities of antibodies induced by vaccination with the compositions described above. The kit of the invention includes peptides of Epitopes I through IV, and coated solid supports, a labelled reagent for detecting the binding of antibodies to these peptides, and miscellaneous substrates and apparatus for evoking or detecting the signals provided by the labels, as well as conventional apparatus for taking blood samples, appropriate vials and other diagnostic assay components.

In yet a further aspect, the invention provides a method for detecting the titers and reactivity patterns of antibodies in subjects vaccinated with the compositions of this invention. The method includes the steps of incubating dilutions of the subject's biological fluid, e.g. serum, with plates or beads on which are bound one or more peptides of the Epitopes I through IV, washing away unbound biological materials, and measuring any antibody binding to the peptides with labeled reagent, e.g., an anti-human immunoglobulin to which is associated an enzyme. Depending on the type of label employed, the signal produced by the label may be evoked by further adding a substrate which reacts with the enzyme, e.g., producing a color change. Other conventional labels may also be incorporated into this assay design.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates an HIV-1 Tat protein consensus sequence (SEQ ID NO: 1), based on Tat protein sequences of 31 known HIV-1 strains found in the common B subtype (NIH Los Alamos database). The amino acid positions in which variations appear are in lower case letters.

FIGS. 2A–2C illustrate a synthetic gene which encodes a fusion protein (SEQ ID NO: 3) of this invention, described in detail in Example 5 below.

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reported in Table 1 below. Dark shading shows self-reactivity, pale shading shows significant (40%) cross-reactivity.

TABLE 1

Immunizing Peptides	Detector Peptides (GMT (% self-binding))			
Position Y	Arg	Lys	Ser	Asn
Arg	77,000 (100)	10,000 (13)	10,000 (13)	9,000 (12)
Lys*	51,000 (62)	82,000 (100)	35,000 (43)	45,000 (65)
Ser	8,000 (6)	8,000 (6)	126,000 (100)	14,000 (11)
Asn*	17,000 (13)	12,000 (9)	61,000 (46)	133,000 (100)

*Only one high titer antiserum available.

Preferably a composition of this invention contains one or more the following Epitope I peptides or polypeptides:

R1-Val-Asp-Pro-Arg-Leu-Glu-Pro-R2 (SEQ ID NO: 6);

R1-Val-Asp-Pro-Lys-Leu-Glu-Pro-R2 (SEQ ID NO: 7);

R1-Val-Asp-Pro-Ser-Leu-Glu-Pro-R2 (SEQ ID NO: 8);

R1-Val-Asp-Pro-Asn-Leu-Glu-Pro-R2 (SEQ ID NO: 9).

As demonstrated above, the immunogen in which Y is Lys (SEQ ID NO: 7) induces antibodies with good reactivity with the three other variants. No immunogen induced high titer antibodies with good cross-reactivity with the variant in which Y was Ser. Thus an immunogen of Epitope I in which Y was Lys (SEQ ID NO: 7) may suffice for full cross-reactivity to all four position Y variants, and may be used alone in an immunogenic composition. While this pattern of response of the peptide in which Y is Lys occurs in the majority of tests to date, it should be expected by one of skill in the art, that some differences in cross-reactivity from the results above may occur in some test samples.

Alternatively, compositions of this invention comprise two, three or all four of these amino acid sequences (SEQ ID NOS: 6–9). Alternatively, a combination of Epitope I immunogens in which Y was Lys and in which Y was Asn (SEQ ID NO: 6) should provide somewhat better titers for Epitope I variants in which Y was Ser or Y was Asn.

Still another alternative Epitope I peptide immunogen contains at position Y an ornithine, since the ornithine side chain is similar to lysine with one less $-CH_2-$. This Epitope I sequence may provide even more cross-reactivity, and may be used alone or in combination with other Epitope I immunogens.

According to the formula of Epitope I above, the seven amino acid residues which form the minimum reactive Epitope I sequences, may be flanked by other amino acids, so that the entire Epitope I sequence may be between 7 and about 25 amino acids in length. As indicated in Example 1 below, the identity of the flanking amino acids is not essential to the biological function of the Epitope I immunogen. In particular additional amino acids on the N-terminus of Epitope I sequences do not affect immunogenicity. Thus, the N-terminal R1 of Epitope I may be selected from the group consisting of a free N terminal amino acid hydrogen, a lower alkyl (i.e., C1–C10 alkyl), a lower C1–C10 alkanoyl, such as an acetyl group, or a sequence of between 1 to about 5 amino acids. Preferably, R1 represents 2 amino acids.

Additional amino acids on the C-terminus of the Epitope I minimum sequence enhance antibody titer. Epitope I immunogens require at least two amino acid extensions at the C-terminus for optimal immunogenicity and are immunogenic when present within extended amino acid sequence. Thus, while the C-terminal R2 can be a simple free hydroxyl group, it can also be a C terminal amide. However, to enhance titer, R2 is preferably a sequence of between 1 to about 14 additional amino acids amidated at the carboxyl terminus. In a preferred embodiment, R2 is -Trp-Lys-His-Pro-Gly-Ser-amide (SEQ ID NO: 10).

The above-described Epitope I composition of the invention may contain a number of additional peptides or polypeptides, which contain other sequences which correspond to amino acid residues between aa 2-aa10 of SEQ ID NO: 1, but are derived from other Tat variants which do not cross-react well with antibodies to the Epitope I compositions described above. These additional peptides and polypeptides are referred to as "optional Epitope Ia immunogens". For example, optional Epitope Ia immunogens which can be present in compositions of this invention, can contain at least one copy of at least one of the following amino acid sequences (SEQ ID NOS: 11 through 18, respectively):

R1-Gly-Pro-Arg-Leu-Glu-Pro-R2;

R1-Ala-Pro-Arg-Leu-Glu-Pro-R2;

R1-His-Pro-Arg-Leu-Glu-Pro-R2;

R1-Asp-Pro-Gly-Leu-Glu-Pro-R2;

R1-Asp-Pro-Arg-Ile-Glu-Pro-R2;

R1-Asp-Pro-Arg-Leu-Gly-Pro-R2;

R1-Asp-Pro-Arg-Leu-Glu-Ala-R2; and

R1-Asn-Pro-Ser-Leu-Glu-Pro-R2.

The Epitope I compositions of this invention may contain multiple copies of a single peptide, or multiple copies of different Epitope I peptides, including optionally Epitope Ia peptides, in any order, or multiple copies of at least two of these peptides. In one embodiment, at least one copy of all four amino acid sequences (SEQ ID NOS: 6–9) are present.

As described in more detail below, the Epitope I and Ia peptides or polypeptides of these compositions are produced synthetically or recombinantly. The Epitope I immunogens can be expressed as synthetic peptides coupled to a carrier protein. The Epitope I immunogens may also be expressed as multiple antigenic peptides, optionally coupled to a carrier protein. Alternatively, the Epitope I immunogens may be expressed within recombinantly produced protein, optionally co-expressed or fused in frame with a carrier protein.

Epitope I compositions demonstrate a biological activity of inducing in an immunized, immune competent mammal, i.e., a non-infected human, or an asymptomatic infected human, an active humoral immune response (i.e., antibodies) that is directed against greater than 95%, and preferably greater than 99%, of the known variants of Tat proteins of HIV-1. The end result of such treatment is an impairment of the multiplication of HIV-1 in an acute infection, thereby preventing high post-seroconversion plasma levels of HIV-1 that are associated with progression to AIDS. Active induction of antibodies in the early asymptomatic phase of HIV infection may reduce viral multiplication, lower the plasma viral load and reduce the likelihood of progression to AIDS. The composition which contains at least one Epitope I immunogen up to all four of the SEQ ID NO: 6–9 amino acid sequences, can elicit an immune response to about 97% of the 400 known Tat sequences of the common B subtypes of HIV-1 and with Tat proteins of all 18 non-B subtype HIV-1 that have been sequenced (courtesy of Dr. Esther Guzman, Los Alamos NIAID HIV database, GenBank database).

B. Epitope II Immunogenic Compositions

In another embodiment, the present invention provides a composition comprising at least one Epitope II amino acid

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sequence. This Epitope II sequence elicits a specific humoral immune response (for the purpose of this invention) in a mammal exposed to the Epitope II sequence in vivo. Epitope II defines peptides of the formula R3-Lys-X-Leu-Gly-Ile-Ser-Tyr-Gly-Arg-Lys-Lys-R4, wherein X is Gly (70%) or Ala (30%). This sequence is highly conserved. The immunogen in which X is Gly induces antibodies cross-reactive with the sequence in which X is Ala.

The N terminal R3 may represent the hydrogen on the unmodified N terminal amino acid Lys, or R3 may be a lower alkyl, or a lower alkanoyl, such as an acetyl group, substituent on the Lys. R3 may also include a sequence of between 1 to about 5 amino acids, optionally substituted with a lower alkyl or lower alkanoyl. The C terminal R4 may represent the free hydroxyl of the C terminal amino acid Lys, or R4 may be an amide on that C terminal amino acid. R4 may optionally be a sequence of one or up to about 5 additional amino acids, optionally substituted with an amide. The presently preferred immunogen for Epitope II is -Lys-Gly-Leu-Gly-Ile-Ser-Tyr-Gly-Arg-Lys-Lys- (amino acids 41–51 of SEQ ID NO: 1). This would react/cross-react with greater than 96% of known HIV-1 Tat proteins.

Epitope II is poorly immunogenic when presented within other sequences. Thus, for optimal immunogenicity, this sequence is prepared as a synthetic peptide fused to, or coupled to, a carrier protein or as a multiple antigenic peptide, optionally coupled to carrier protein. Alternatively, Epitope II may be expressed as the C terminal sequence of a recombinant protein, which is optionally fused in frame to a carrier protein at its amino terminal sequence. In a composition of this invention, an Epitope II peptide is preferably presented alone or in combination with one or more Epitope I peptides. Other compositions may employ one or more Epitope III or IV peptides.

C. Epitope III Immunogenic Compositions

In another embodiment, the present invention provides a composition comprising at least one, and preferably two or more Epitope III amino acid sequences. These Epitope III sequences elicit a specific humoral immune response (for the purpose of this invention) in a mammal exposed to the Epitope III sequences in vivo. This epitope shows considerable more variation than Epitopes I and II. These Epitope III immunogenic peptides and polypeptides are derived from Tat variant protein sequences corresponding to amino acids 56–62 of SEQ ID NO: 1. Epitope III defines peptides of the formula: R5-Arg-Arg-X-Z-A-Y-Ser-R6 (SEQ ID NO: 38), wherein X may be Ala, Pro, Ser or Gln; Y may be Asp, Asn, Gly or Ser; Z may be Pro or His; and A may be Gin or Pro. The Epitope III immunogens in which X is Ala induce antibodies that cross-react with the other position X variants. Epitope III immunogens containing Asp in position Y induce antibodies that cross-react with the other position Y variants. The three most common variants for positions Z and A are -Pro-Gln- (61%), -Pro-Pro- (8%) and -His-Gln- (8%). Antibodies induced by these three immunogens do not cross-react with the others so that three immunogens would need to be used to cover these variants (77%).

According to the formula of Epitope III above, the seven amino residues which form the minimum reactive Epitope III sequences, may be flanked by other amino acids, so that the entire Epitope III sequence may be between 7 and about 15 amino acids in length. As indicated in Example 3 below, the identity of the flanking amino acids is not essential to the biological function of the Epitope III immunogen. In particular additional amino acids on the N-terminus of Epitope III sequences do not affect immunogenicity. The N terminal R5 may optionally represent the hydrogen on the N-terminal Arg, or R5 is a lower alkyl or alkanoyl, such as an acetyl group, substituent on the N-terminal Arg. Alternatively, R5 is a sequence of between 1 to about 3 amino acids, optionally substituted with a lower alkyl or lower alkanoyl. In a preferred embodiment R5 is -Gln-Arg-, optionally modified as above, which improves the immunogenicity of the Epitope. The C terminal R6 represents either the free hydroxyl on the C terminal amino acid or an amide substituent on the C terminal amino acid, because any C-terminal extension impairs immunogenicity.

Epitope III immunogens which can be present in compositions of this invention can include at least one copy of at least one of the following preferred Epitope III amino acid sequences: R5-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser-R6, R5-Gln-Arg-Arg-Arg-Ala-His-Gln-Asp-Ser-R6, and R5-Gln-Arg-Arg-Arg-Ala-Pro-Pro-Asp-Ser-R6, optionally modified as above (SEQ ID NOS: 20 through 22, respectively).

Still other optional immunogenic sequences which may be included in the Epitope III compositions include R5-Arg-Arg-Pro-Pro-Gln-Asp-Asn-R6, R5-Arg-Arg-Ala-Pro-Gln-Asp-Arg-R6, R5-Arg-Gly-Ala-Pro-Gln-Asp-Ser-R6; R5-Arg-Arg-Ala-Pro-Glu-Asp-Ser-R6; or R5-Arg-Arg-Ala-Ser-Gln-Asp-Ser-R6 (SEQ ID NOS: 23 through 27, respectively). As can be determined from review of the examples below, the inclusion of these Epitope III peptides in compositions of the invention can induce antibodies that react with rare Tat proteins of HIV-1 which are not cross-reactive with, or do not have a sufficiently strong cross-reactivity to, antibodies induced by the preferred Epitope III immunogens.

As described in more detail below, the Epitope III peptides or polypeptides are poorly immunogenic when presented within other sequences. Although the Epitope III sequences may be prepared recombinantly, for optimal immunogenicity, these sequences would be prepared synthetically and coupled to a carrier protein, or as multiple antigenic peptides, optionally coupled to carrier protein. Alternatively, Epitope III may be expressed as the C terminal sequence of a recombinant protein, which is optionally fused in frame to a carrier protein at its amino terminal sequence. Compositions of this invention would preferably contain three or more different Epitope III immunogens, optionally with at least one Epitope I immunogen, and optionally with one or more Epitope II or Epitope IV immunogens.

D. Epitope IV Immunogenic Compositions

In another embodiment, the present invention provides a composition comprising at least one, and preferably two or more Epitope IV amino acid sequences. These Epitope IV sequences elicit a specific humoral immune response (for the purpose of this invention) in a mammal exposed to the Epitope IV sequences in vivo. The Epitope IV immunogenic peptides and polypeptides are derived from Tat variant protein sequences corresponding to amino acids 62–72 of SEQ if) NO: 1, including a C-terminal Pro from Exon 2 of HIV-1 Tat. Epitope IV defines peptides of the formula: R7-Ser-Gln-X-His-Gln-Y-Ser-Leu-Ser-Lys-Gln-Pro-R8 (SEQ ID NO: 39), wherein X may be Asn or Thr; and Y may be Ala or Val. The immunogen in which X is Thr induces antibodies that cross-react with the immunogen in which X is Asn. The immunogen in which Y is Val induce antibodies that do not cross-react with the peptides in which Y is Ala. However, the peptides containing Ala in position Y induce antibodies that cross-react with peptides for Epitope IV in which Y is Val. Thus the optimal Epitope IV immunogen is Ser-Gln-Thr-His-Gln-Ala-Ser-Leu-Ser Lys-Gln-Pro (SEQ

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ID NO: 40) and this induces antibodies reactive/cross-reactive with 64% of known HIV-1 Tat proteins.

According to the formula of Epitope IV above, the twelve amino residues which form the minimum reactive Epitope IV sequences, may be flanked by a few other amino acids, so that the entire Epitope IV sequence may be between 12 and about 18 amino acids in length. The N terminal R7 may represent the hydrogen of the N terminal amino acid, or a lower alkyl or alkanoyl, such as an acetyl group, substituent on the N terminal amino acid. Although N-terminal extension markedly inhibits immunogenicity, the R7 may also be a sequence of between 1 to about 3 amino acids, optionally substituted with a lower alkyl or lower alkanoyl. The C terminal R8 may represent the free hydroxyl on the C terminal amino acid, or an amide substituent on the C terminal amino acid, or R8 may be a sequence of one or up to about 3 additional amino acids, optionally substituted with an amide. Additionally, the C-terminal Pro, which is an important component of the epitope, is encoded by Exon 2 of Tat. Thus antibodies to Epitope IV would be poorly reactive with non-spliced Tat Exon 1 protein.

This Epitope IV sequence is poorly immunogenic when presented within other sequences, Thus, for optimal immunogenicity, this sequence would be prepared as a synthetic peptide coupled to carrier protein or as a multiple antigenic peptide, optionally coupled to carrier protein. Compositions of this invention would preferably contain two or more different Epitope IV immunogens, optionally with at least one Epitope I immunogen, and optionally with one or more Epitope II or Epitope III immunogens.

E. Compositions Containing Multiple Epitopes

While the amino acid sequences of Epitope I, II, III and IV and optional immunogens identified herein were obtained by rigorous analysis of over 400 known Tat sequences of HIV-1, it should be understood by one of skill in the art that similar compositions may be obtained, following the teachings of this invention, from the study of further Tat proteins, the nucleic acid sequences encoding them, and fragments thereof from newly isolated Tat proteins of HIV-1 subtype B, or from Tat proteins of the other subtypes, or from other HIV strains.

Thus, the compositions of this invention, i.e., the peptide/polypeptides containing the above-identified amino acid sequences, when provided to a human subject, are useful in the immunologic interdiction of extracellular Tat proteins of most HIV-1 strains. These compositions function to critically reduce explosive multiplication of the virus and permit effective immune control of the virus.

The immunogens for each Epitope are preferably designed to induce antibodies reactive with the highest proportion of naturally occurring variants of each epitope. For an epitope such as Epitope I, multiple copies of an immunogen could be incorporated in a synthetic or recombinant immunogen to enhance the immunogenicity and produce higher titer antibodies. Furthermore, immunogens for two or more epitopes could be combined to extend coverage, since variations in sequence of each epitope occur independently. For example, combining an Epitope I immunogen(s) (95%) with an Epitope II immunogen (96%) would result in antibodies in immunologically responsive subjects reactive with 99.8% of known HIV-1 Tat proteins. Thus, as one example, a composition of this invention contains one Epitope I (underlined)-Epitope II (double-underlined) fused peptide immunogen such as Cys-Glu-Pro-Val-Asp-Pro-Lys-Leu-Glu-Pro-Trp-Lys-Glu-Leu-Gly-Ile-Ser-Tyr-Gly-Arg-Lys-Lys-amide (SEQ ID NO: 67), coupled to carrier protein attached to Epitope I, or the same peptide (less the N-terminal Cys for coupling), synthesized as a multiple antigenic peptide, optionally coupled to a carrier protein. Alternatively, mixtures of two or more immunogens could be used as follows.

The Epitope I immunogens, with or without any Epitope II, III or IV or other optional immunogens, may be prepared and used in immunogenic compositions in a variety of forms, for example, chemically synthesized or as recombinant peptides, polypeptides, proteins, fusion proteins or fused peptides.

1. Synthetic Peptide/Protein Coupled to a Carrier

As one embodiment, a composition of the present invention may be a synthetic peptide, containing single or multiple copies of the same or different Epitope I immunogen amino acid sequences and/or Epitope II/III/IV immunogenic amino acid sequences, and optionally amino acid sequences of the optional immunogens, coupled to a selected carrier protein. In this embodiment of a composition of this invention, multiple above-described Epitope I amino acid sequences with or without flanking sequences, may be combined sequentially in a polypeptide and coupled to the same carrier. Alternatively, the Epitope I, II, III, or IV immunogens, may be coupled individually as peptides to the same or different carrier proteins, and the resulting immunogen-carrier constructs mixed together to form a single composition.

For this embodiment, the carrier protein is desirably a protein or other molecule which can enhance the immunogenicity of the selected immunogen. Such a carrier may be a larger molecule which has an adjuvanting effect. Exemplary conventional protein carriers include, without limitation, *E. coli* DnaK protein, galactokinase (galK, which catalyzes the first step of galactose metabolism in bacteria), ubiquitin, α-mating factor, β-galactosidase, and influenza NS-1 protein. Toxoids (i.e., the sequence which encodes the naturally occurring toxin, with sufficient modifications to eliminate its toxic activity) such as diphtheria toxoid and tetanus toxoid may also be employed as carriers. Similarly a variety of bacterial heat shock proteins, e.g., mycobacterial hsp-70 may be used. Glutathione reductase (GST) is another useful carrier. One of skill in the art can readily select an appropriate carrier.

In particularly desirable immunogen-carrier protein construct, one or more epitope immunogen and optional immunogen peptides/polypeptides may be covalently linked to a mycobacterial *E. coli* heat shock protein 70 (hsp70) (K. Suzue et al, J. Immunol., 156:873 (1996)). In another desirable embodiment, the composition is formed by covalently linking the immunogen-containing peptide or polypeptide sequences to diphtheria toxoid.

2. Multiple Antigenic Peptide

In yet another embodiment, the peptides or polypeptide epitope immunogens and any selected optional immunogens may be in the form of a multiple antigenic peptide ("MAP", also referred to as an octameric lysine core peptide) construct. Such a construct may be designed employing the MAP system described by Tam, Proc. Natl. Acad. Sci. USA, 85:5409–5413 (1988). This system makes use of a core matrix of lysine residues onto which multiple copies of the same Epitope I or optional immunogens of the invention are synthesized as described (D. Posnett et al., J. Biol. Chem., 263(4):1719–1725 (1988); J. Tam, "Chemically Defined Synthetic Immunogens and Vaccines by the Multiple Antigen Peptide Approach", Vaccine Research and Developments, Vol. 1, ed. W. Koff and H. Six, pp. 51–87 (Marcel Deblau, Inc., New York 1992)). Each MAP contains multiple copies of only one peptide. Therefore, e.g., an

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epitope composition of this invention can include a MAP in which the peptide or polypeptide epitope immunogen attached to the lysine core contains one or sequential repeats of the four amino acid sequences (SEQ ID NOS: 6–9) identified above. Multiple different MAPs may be employed to obtain any desired combination of Epitope I, II, III or IV sequences. Preferably these MAP constructs are associated with other T cell stimulatory sequences, or as pharmaceutical compositions, administered in conjunction with T cell stimulatory agents, such as known adjuvants.

3. Spacers

In either of the above compositions, e.g., as peptide/polypeptide-carrier constructs or MAPs, each peptide/polypeptide immunogen, or each amino acid sequence in the immunogen, may be optionally separated by optional amino acid sequences called "spacers". Spacers are sequences of between 1 to about 4 amino acids which are interposed between two sequences to permit linkage therebetween without adversely effecting the three dimensional structure of the immunogen. Spacers may also contain restriction endonuclease cleavage sites to enable separation of the sequences, where desired. Suitable spacers or linkers are known and may be readily designed and selected by one of skill in the art. Preferred spacers are sequences containing Gly and/or Ser amino acids.

F. Nucleic Acid Compositions of the Invention, Including a Synthetic Gene

Other embodiments of this invention include nucleic acid sequences, which encode the above-described peptide/polypeptide compositions, including the peptide and polypeptide immunogens of the compositions described above, including those peptides and polypeptides fused to carrier proteins. The nucleic acid sequences may also include sequences encoding the carrier proteins.

Thus, one preferred embodiment of the invention is a "synthetic gene" which encodes sequentially for one or more Epitope I immunogenic peptides/polypeptide. The synthetic gene preferably encodes two, three or all four Epitope I amino acid sequences (SEQ ID NOS: 6 through 9, respectively):

R1-Val-Asp-Pro-Arg-Leu-Glu-Pro-R2;

R1-Val-Asp-Pro-Lys-Leu-Glu-Pro-R2;

R1-Val-Asp-Pro-Ser-Leu-Glu-Pro-R2; and

R1-Val-Asp-Pro-Asn-Leu-Glu-Pro-R2.

The synthetic gene can also encode any selection of the optional immunogens identified above, and may include an Epitope II or III immunogen provided that the Epitope II or III peptide is fused to the C terminus of the Epitope I sequence and not further modified on its own C terminus. The synthetic gene may encode multiple copies of the same amino acid sequence, copies of multiple different immunogens or amino acid sequences, or multiple copies of multiple different immunogens or amino acid sequences. The synthetic gene may encode the selected amino acid sequences in an open reading frame with, or fused to, a nucleic acid sequence encoding a carrier protein. A further characteristic of the synthetic gene may be that it encodes a spacer between each sequence encoding an immunogen and/or between the sequence encoding an immunogen and the sequence encoding the carrier protein.

The synthetic gene of the present invention may also be part of a synthetic or recombinant molecule. The synthetic molecule may be a nucleic acid construct, such as a vector or plasmid which contains the synthetic gene encoding the protein, peptide, polypeptide, fusion protein or fusion peptide under the operative control of nucleic acid sequences encoding regulatory elements such as promoters, termination signals, and the like. Such synthetic molecules may be used to produce the polypeptidelpeptide immunogen compositions recombinantly.

The synthetic gene or synthetic molecules can be prepared by the use of chemical synthesis methods or preferably, by recombinant techniques. For example, the synthetic gene or molecules may contain certain preference codons for the species of the indicated host cell.

The synthetic gene or molecules, preferably in the form of DNA, may be used in a variety of ways. For example, these synthetic nucleic acid sequences may be employed to express the peptide/polypeptides of the invention in vitro in a host cell culture. The expressed immunogens, after suitable purification, may then be incorporated into a pharmaceutical reagent or vaccine.

Alternatively, the synthetic gene or synthetic molecule of this invention may be administered directly into a mammalian, preferably human subject, as so-called 'naked DNA' to express the protein/peptide immunogen in vivo in a patient. See, e.g., J. Cohen, Science, 259:1691–1692 (Mar. 19, 1993); E. Fynan et al., Proc. Natl. Acad. Sci., USA, 90:11478–11492 (Decemeber 1993);and J. A. Wolffet al., Biotechniques, 11:474–485 (1991), all incorporated by reference herein. The synthetic molecule, e.g., a vector or plasmid, may be used for direct injection into the mammalian host. This results in expression of the protein by host cells and subsequent presentation to the immune system to induce antibody formation in vivo.

G. Microorganisms that Express the Synthetic Gene

In still another aspect of the present invention, the synthetic genes or molecules of this invention may be incorporated into a non-pathogenic microorganism. The resulting microorganism, when administered to a mammalian host expresses and multiplies the expressed compositions of this invention in vivo to induce specific antibody formation. For example, non-pathogenic recombinant viruses or commensal bacterium which carry the compositions or synthetic genes of this invention and are useful for administration to a mammalian patient may be prepared by use of conventional methodology and selected from among known non-pathogenic microorganisms.

Among commensal bacterium which may be useful for exogenous delivery of the synthetic molecule to the patient, and/or for carrying the synthetic gene into the patient in vivo, include, for example, various strains of Streptococcus, e.g., *S. gordonii*, or *E. coli*, Bacillus, Streptomyces, and Saccharomyces.

Suitable non-pathogenic viruses which may be engineered to carry the synthetic gene into the cells of the host include poxviruses, such as vaccinia, adenovirus, canarypox, retroviruses and the like. A number of such non-pathogenic viruses are commonly used for human gene therapy, and as carrier for other vaccine agents, and are known and selectable by one of skill in the art.

H. Preparation or Manufacture of Compositions of the Invention

The compositions of the invention, and the individual polypeptides/peptides containing the Epitope I, II, III or Epitope IV or optional immunogens of this invention, the synthetic genes, and synthetic molecules of the invention, may be prepared conventionally by resort to known chemical synthesis techniques, such as described by Merrifield, J. Amer. Chem. Soc., 85:2149–2154 (1963). Alternatively, the compositions of this invention may be prepared by known recombinant DNA techniques by cloning and expressing within a host microorganism or cell a DNA fragment carrying a sequence encoding a peptide/polypeptide containing

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an Epitope I and/or optional immunogen and optional carrier protein. Coding sequences for the Epitope I and optional immunogens can be prepared synthetically (W. P. C. Stemmer et al, Gene, 164:49 (1995) or can be derived from viral RNA by known techniques, or from available cDNA-containing plasmids.

Combinations of these techniques may be used, such as for production of the synthetic gene, which may require assembly of sequential immunogens by conventional molecular biology techniques, and site-directed mutagenesis to provide desired sequences of immunogens. The product of the synthetic gene is then produced recombinantly. All of these manipulations may be performed by conventional methodology.

Systems for cloning and expressing the peptide/polypeptide compositions of this invention using the synthetic genes or molecules, include various microorganisms and cells which are well known in recombinant technology. These include, for example, various strains of *E. coli*, Bacillus, Streptomyces, and Saccharomyces, as well as mammalian, yeast and insect cells. Suitable vectors therefor are known and available from private and public laboratories and depositories and from commercial vendors. Currently, the most preferred host is a mammalian cell such as Chinese Hamster ovary cells (CHO) or COS-1 cells. These hosts may be used in connection with poxvirus vectors, such as vaccinia or swinepox. The selection of other suitable host cells and methods for transformation, culture, amplification, screening and product production and purification can be performed by one of skill in the art by reference to known techniques. See, e.g., Gething and Sambrook, Nature, 293:620–625 (1981).

Another preferred system includes the baculovirus expression system and vectors.

When produced by conventional recombinant means, the compositions of this invention, i.e., the polypeptide/peptides containing the indicated copies of the Epitope I immunogens and optional immunogens may be isolated either from the cellular contents by conventional lysis techniques or from cell medium by conventional methods, such as chromatography. See, e.g., Sambrook et al., Molecular Cloning. A Laboratory Manual., 2d Edit., Cold Spring Harbor Laboratory, New York (1989).

Suitable plasmid and viral vectors used either for production of the peptide/polypeptide components as DNA vaccines are well known to those of skill in the art and are not a limitation of the present invention. See, Sambrook et al., cited above and the references above to production of the protein. See, also International Patent Publication No. WO94/01139, published Jan. 20, 1994.

Briefly, the DNA encoding the selected peptide/polypeptide is inserted into a vector or plasmid which contains other optional flanking sequences, a promoter, an mRNA leader sequence, an initiation site and other regulatory sequences capable of directing the multiplication and expression of that sequence in vivo or in vitro. These vectors permit infection of patient's cells and expression of the synthetic gene sequence in vivo or expression of it as a protein/peptide or fusion protein/peptide in vitro.

The resulting composition may be formulated into a Epitope I composition with any number of optional immunogens and screened for efficacy by in vivo assays. Such assays employ immunization of an animal, e.g., a rabbit or a simian, with the composition, and evaluation of titers of antibody to the Tat proteins of HIV-1 or to synthetic detector peptides corresponding to variant Tat sequences (as shown in the examples below).

I. Antibody Compositions of the Invention

An isolated mammalian antibody composition which is directed against a peptide or polypeptide of the invention, as described above, is also an aspect of this invention. Such polyclonal antibody compositions are produced by immunizing a mammal with a peptide/polypeptide composition containing an assortment of Epitope I, II, III, and/or IV immunogens and optional immunogens, as described above. Suitable mammals include primates, such as monkeys; smaller laboratory animals, such as rabbits and mice, as well as larger animals, such as horse, sheep, and cows. Such antibodies may also be produced in transgenic animals. However, a desirable host for raising polyclonal antibodies to a composition of this invention includes humans.

The polyclonal antibodies raised in the mammal exposed to the composition are isolated and purified from the plasma or serum of the immunized mammal by conventional techniques. Conventional harvesting techniques can include plasmapheresis, among others.

Such polyclonal antibody compositions may themselves be employed as pharmaceutical compositions of this invention. Alternatively, other forms of antibodies may be developed using conventional techniques, including monoclonal antibodies, chimeric antibodies, humanized antibodies and fully human antibodies. See, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988); Queen et al., Proc. Nat'l. Acad. Sci. USA, 86:10029–10032 (1989); Hodgson et al., Bio/Technology, 9:421 (1991); International PCT Application PCT/GB91/01554, Publication No. WO92/04381 and International PCT Application PCT/GB93/00725, Publication No. WO93/20210). Other anti-Tat antibodies may be developed by screening hybridomas or combinatorial libraries, or antibody phage displays (W. D. Huse et al., Science, 246:1275–1281 (1988)) using the polyclonal or monoclonal antibodies produced according to this invention and the amino acid sequences of the Epitope I, II, I, IV or optional immunogens.

These antibody compositions bind to greater than 95%, and preferably greater than 99% of known Tat protein variants of HIV-1, and prevent the Tat proteins from supporting further HIV-1 multiplication. Thus, these antibodies are useful in pharmaceutical methods and formulations described below.

J. Pharmaceutical Compositions of the Invention

As another aspect of this invention, a pharmaceutical composition useful for inducing antibodies that react with greater than 95%, preferably greater than 99%, of known HIV-1 Tat proteins and impair the multiplication of HIV-1 can comprise as its active agents, one or more of the peptides or polypeptides of Epitope I, II, III, or IV. Several desirable compositions include the following above-described components:

(a) a peptide/polypeptide immunogen which contains at least one, and preferably all four of the Epitope I amino acid sequences SEQ ID NOS: 6–9;

(b) a peptide/polypeptide immunogen which contains at least one of the Epitope II amino acid sequences;

(c) a peptide/polypeptide immunogen which contains at least one, and preferably three Epitope III amino acid sequences;

(d) a peptide/polypeptide immunogen which contains at least one Epitope IV amino acid sequence;

(e) a synthetic gene encoding one or more of Epitope I, Epitope II, Epitope III, or Epitope IV sequences as described above;

(f) a synthetic molecule described above;

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(g) a recombinant virus carrying the synthetic gene or molecule; and

(h) a commensal bacterial carrying the synthetic gene or molecule.

The selected active component(s) is present in a pharmaceutically acceptable carrier, and the composition may contain additional ingredients. Pharmaceutical formulations containing the compositions of this invention may contain other active agents, such as T cell stimulatory agents for the MAPs, adjuvants and immunostimulatory cytokines, such as IL-12 and other well-known cytokines, for the protein/peptide compositions. All of these pharmaceutical compositions can operate to lower the viral levels of a mammal.

As pharmaceutical compositions, these compositions comprising Epitope I and/or II, and/or III, and/or IV amino acid sequences with optional immunogenic amino acid sequences are admixed with a pharmaceutically acceptable vehicle suitable for administration as a protein composition for prophylaxis or treatment of virus infections. These proteins may be combined in a single pharmaceutical preparation for administration. Suitable pharmaceutically acceptable carriers for use in an immunogenic proteinaceous composition of the invention are well known to those of skill in the art. Such carriers include, for example, saline, buffered saline, a selected adjuvant, such as aqueous suspensions of aluminum and magnesium hydroxides, liposomes, oil in water emulsions and others. Suitable adjuvants may also be employed in the protein-containing compositions of this invention. The present invention is not limited by the selection of the carrier or adjuvant.

Suitable vehicles for direct DNA, plasmid nucleic acid, or recombinant vector administration include, without limitation, saline, or sucrose, protamine, polybrene, polylysine, polyrations, proteins, $CaPO_4$ or spermidine. See e.g, International Patent Publication No. WO94/01139 and the references cited above.

The peptide/polypeptide compositions and synthetic genes or molecules in vivo are capable of eliciting in an immunized host mammal, e.g., a human, an immune response capable of interdicting greater than about 95 to about 99% of known extracellular Tat protein variants from HIV-1 and thereby lowering the viral levels.

Yet another pharmaceutical composition useful for impairing the multiplication of HIV-1 comprises an antibody composition as described in detail above. In a pharmaceutical composition, the antibodies may be carried in a saline solution or other suitable carrier. The antibody compositions are capable of providing an immediate, exogenously provided interdiction of Tat.

The preparation of these pharmaceutically acceptable compositions, from the above-described components, having appropriate pH isotonicity, stability and other conventional characteristics is within the skill of the art.

K. Method of the Invention-Impairing Multiplication of HIV-1

According to the present invention, a method for reducing the viral levels of HIV-1 involves exposing a human to the Tat antibody-inducing pharmaceutical compositions described above, actively inducing antibodies that react with greater than 95%, preferably greater than 990%, of known HIV-1 Tat proteins, and impairing the multiplication of the virus in vivo. This method is appropriate for an HIV-1 infected subject with a competent immune system, or an uninfected or recently infected subject. The method induces antibodies which react with HIV-1 Tat proteins, which antibodies reduce viral multiplication during any initial acute infection with HIV-1 and minimize chronic viremia leading to AIDS. This method also lowers chronic viral multiplication in infected subjects, again minimizing progression to AIDS.

In one embodiment, the pharmaceutical compositions may be therapeutically administered to an HIV-1 infected human with a competent immune system for treatment or control of viral infection. Such an infected human may be asymptomatic. In a similar embodiment, the pharmaceutical compositions may be administered to an uninfected human for prophylaxis.

In these two instances, the pharmaceutical compositions preferably contain the peptide/polypeptide compositions, the synthetic genes or molecules, the recombinant virus or the commensal recombinant bacterium. Each of these active components of the pharmaceutical composition actively induces in the exposed human the formation of anti-Tat antibodies which block the transfer of Tat from infected cells to other infected or uninfected cells. This action reduces the multiplicity of infection and blocks the burst of HIV-1 viral expansion, and thus lowers viral levels. In already infected patients, this method of reduction of viral levels can reduce chronic viremia and progression to AIDS. In uninfected humans, this administration of the compositions of the invention can reduce acute infection and thus minimize chronic viremia leading to progression to AIDS.

Yet another aspect of the invention is a method for reducing the viral levels of HIV-1 by administering to a human, who is incapable of mounting an effective or rapid immune response to infection with HIV-1, a pharmaceutical composition containing the antibody compositions described above. The method can involve chronically administering the composition. Among such patients suitable for treatment with this method are HIV-1 infected patients who are immunocompromised by disease and unable to mount a strong immune response. In later stages of HIV infection, the likelihood of generating effective titers of antibodies is less, due to the immune impairment associated with the disease. Also among such patients are HIV-1 infected pregnant women, neonates of infected mothers, and unimmunized patients with putative exposure (e.g., a human who has been inadvertently "stuck" with a needle used by an HIV-1 infected human).

For such patients, the method of the invention preferably employs as the pharmaceutical composition the antibody composition of the invention, which is a polyclonal antibody composition prepared in other mammals, preferably normal humans. Alternatively, the other forms of antibody described above may be employed. These antibody compositions are administered as passive immunotherapy to inhibit viral multiplication and lower the viral load. The exogenous antibodies which react with greater than 95%, preferably greater than 99%, of known Tat proteins from HIV-1 provide in the patient an immediate interdiction of the transfer of Tat from virally infected cells to other infected or uninfected cells. According to this method, the patient may be chronically treated with the antibody composition for a long treatment regimen.

In each of the above-described methods, these compositions of the present invention are administered by an appropriate route, e.g., by the subcutaneous, oral, intravenous, intraperitoneal, intramuscular, nasal, or inhalation routes. The presently preferred route of administration is intramuscular for the immunizing (active induction) compositions and intravenous or intramuscular for the antibody (passive therapy) compositions. The recombinant viral vectors or naked DNA is preferably administered i.m.; however, other certain recombinant viral vectors and/or live commensal bacteria may be delivered orally.

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-Asp-Pro-Gly-Leu-Glu-Pro- (2) (SEQ ID NO: 14), and single examples of:

-Asp-His-Arg-Leu-Glu-Pro- (SEQ ID NO: 41),

-Ala-Pro-Arg-Leu-Glu-Pro- (SEQ ID NO: 12),

-His-Pro-Arg-Leu-Glu-Pro- (SEQ ID NO: 13),

-Asp-Pro-Arg-Ile-Glu-Pro- (SEQ ID NO: 15),

-Asp-Pro-Arg-Leu-Gly-Pro- (SEQ ID NO: 16),

-Asp-Pro-Arg-Leu-Glu-Ala- (SEQ ID NO: 17) and

-Asn-Pro-Ser-Leu-Glu-Pro- (SEQ ID NO: 18).

For the 18 non-B subtype sequences, 2 had Arg, 1 had Lys, 2 had Ser and 9 had Asn at position 3 of the hexapeptides aa5–10, and other variants were

-Asp-Pro-Asn-Leu-Asp-Pro- (2) (SEQ ID NO: 42) and single examples of

-Asp-Pro-Asn-Ile-Glu-Pro- (SEQ ID NO: 43) and

-Asp-Pro-Asn-Leu-Glu-Ser- (SEQ ID NO: 44).

B. Assessment of Immunological Reactivity and Cross-reactivity of the Four Primary Immunogens

Immunizing and detector sequences were synthesized, as described in Example 1, for the following sequences (SEQ ID NOS: 28 and 45 through 47, respectively):

-Val-Asp-Pro-Arg-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-,

-Val-Asp-Pro-Lys-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-,

-Val-Asp-Pro-Ser-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-,

-Val-Asp-Pro-Asn-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-.

Rabbits were immunized and the antiserums were tested by ELISA, as described in Example 1, for reactivity and cross-reactivity. Self-reactivities are summarized in Table 8.

TABLE 8

Immunogen and detector sequence	GMT	SEQ ID NO
-Val-Asp-Pro-Arg-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-	88,000	28
-Val-Asp-Pro-Lys-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser- Pro-Gly-Ser-	132,000	45
-Val-Asp-Pro-Ser-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-	166,355	46
-Val-Asp-Pro-Asn-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-	173,097	47

Cross-reactivities between these primary immunogens with varying amino acid residues at position 3 of Epitope I are displayed in Table 9. Note that the results reported below are averages with one poorly reactive antiserum.

TABLE 9

	Antiserums to primary immunogens (figures denote % reactivity v. self-reactivity)			
Detectors	Arg3	Lys3	Ser3	Asn3
Arg3	100	49	3	4
Lys3	24	100	6	5
Ser3	11	16	100	15
Asn3	11	22	10	100

Tables 8 and 9 demonstrate that each variant is an effective immunogen, but in general there is only modest cross-reactivity between variants. The best cross-reactivity is obtained with the Lys 3-containing immunogen. This implies that optimal coverage would require inclusion of all four variants as immunogens in a primary composition as described above.

C. Assessment of Cross-reactivities of Other Variants.

Detector peptides were made for all remaining epitope variants and tested for cross-reactivity with the antiserums to the appropriate position 3 primary hexapeptide immunogen. The cross-reactivities versus self-reactivity with the appropriate position 3 primary immunogen are displayed in Table 10.

TABLE 10

Reactivity	Detector Sequence (variations in Epitope I)	% Cross-reactivity	SEQ ID NO:
Cross-reactive	-Asp-His-Arg-Leu-Glu-Pro-	55	41
Cross-reactive	-Asp-Pro-Asn-Ile-Glu-Pro-	70	43
Cross-reactive	-Asp-Pro-Asn-Leu-Asp-Pro-	100	42
Cross-reactive	-Asp-Pro-Asn-Leu-Glu-Ser-	78	44
Non-cross reactive	-Gly-Pro-Arg-Leu-Glu-Pro-	1	11
Non-cross reactive	-Ala-Pro-Arg-Leu-Glu-Pro-	1	12
Non-cross reactive	-His-Pro-Arg-Leu-Glu-Pro-	1	13
Non-cross reactive	-Asp-Pro-Gly-Leu-Glu-Pro-	1	14
Non-cross reactive	-Asp-Pro-Arg-Ile-Glu-Pro-	9	15
Non-cross reactive	-Asp-Pro-Arg-Leu-Gly-Pro-	10	16
Non-cross reactive	-Asp-Pro-Arg-Leu-Glu-Ala-	1	17
Non-cross reactive	-Asn-Pro-Ser-Leu-Glu-Pro-	10	18

The results of Tables 8–10 indicate that immunization with the four primary immunogens would generate antibodies reactive with greater than 97% of HIV-1 Tat proteins of the common B subtype. Interestingly all 18 non-B subtypes in the databases had Epitope I sequences reactive with antibodies to the primary immunogens.

D. Immunogenicity of Certain Epitope I Variants

Immunizing and detecting peptides were synthesized for the following 2 variant Epitope I peptides, with immunizations and ELISA testing as in Example 1. The self titers (GMT) are displayed in Table 11.

TABLE 11

Detecting and Immunizing Peptides (GMT)	Self-titer	SEQ ID NOS.
-Val-Asn-Pro-Ser-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-	94,919	48
-Val-Asp-His-Arg-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-	72,686	49

These data show that inclusion of rare variants along with the primary immunogens expands antibody coverage to such rare epitope variants.

Immunization with the four primary Epitope I sequences can induce high titer antibodies reactive with Tat proteins of >97% of all HIV-1 strains. This coverage can be optionally extended with the inclusion of additional rare Epitope I variant sequences in the immunizing composition.

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extension up to a point enhances immunogenicity, with maximal titers being obtained with Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser (amino acids 54–62 of SEQ ID NO: 1) and a drop in immunogenicity occurring with Arg-Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser- (amino acids 52–62 of SEQ ID NO: 1) as the immunogen. GMT on Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser- (amino acids 53–62 of SEQ ID NO: 1) is reported in Table 16 as % of GMT of antiserums to this peptide on this detector peptide.

TABLE 16

N Term	Immunogen Sequence	C Term	% binding	SEQ ID #
N + 3	Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser-NH_2	C0	100	107
N + 1	Gly-Arg-Arg-Ala-Pro-Gln-Asp-Ser-NH_2	C0	11	108
N0	Arg-Arg-Ala-Pro-Gln-Asp-Ser-Gln-Thr-His-Gln-NH_2	C + 4	3	109
N + 2	Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser-OH	C0	136	(amino acids 54–62 of SEQ ID NO: 1)
N + 3	Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser-Gln-Thr-OH	C + 2	9	(amino acids 53–64 of SEQ ID NO: 1)
N + 3	Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser-Gln-Thr-His-Gln-OH	C + 4	28	(amino acids 53–66 of SEQ ID NO: 1)
N + 4	Arg-Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser-OH	C0	3	(amino acids 52–62 of SEQ ID NO. 1)

EXAMPLE 5

Sequence Variations in Epitope III of HIV-1 Tat Protein and Immunological Cross-reactivities of Antiserums to these Sequences

Variations in the sequence of Tat protein AA 56–72 were analyzed in sequences available in HUMAN RETROVIRUSES and AIDS 1996, (cited above) and additional sequences (GenBank), as described in Example 4.

A. Variations in Epitope III Sequences

482 sequences of Epitope III of the common B subtype of HIV-1 were available for analysis. The most frequent sequence found conformed to the formula -Arg-Arg-X-Pro-Gln-Y-Ser- (SEQ ID NO: 110), where X is Ala, Pro, Ser, or Gln, and Y is Asp, Asn, Gly or Ser. This sequence type, which appears immunologically cross-reactive (see below), was found in 292 of 482 (61%) of available Tat sequences.

Other sequence variants occurred in lower incidence and these included those listed in Table 17 below.

TABLE 17

SEQUENCES	# (%) of Tat Sequences	SEQ ID NO.
-Arg-Arg-Ala-Pro-Pro-Asp-Ser-	20 (4%)	20
-Arg-Arg-Ala-Pro-Pro-Asp-Asn-	21 (4%)	50
-Arg-Arg-Ala-His-Gln-Asp-Ser-	20 (4%)	21
-Arg-Arg-Ala-His-Gln-Asn-Ser-	17 (3.5%)	22
-Arg-Arg-Ala-Pro-Gln-Gly-Asn-	10 (2%)	51
-Arg-Gly-Ala-Pro-Gln-Asp-Ser-	9 (2%)	25
-Arg-Arg-Ala-Pro-Glu-Asp-Ser-	8 (2%)	26
-Arg-Arg-Ala-Ser-Gln-Asp-Ser-	8 (2%)	27

TABLE 17-continued

SEQUENCES	# (%) of Tat Sequences	SEQ ID NO.
-Arg-Arg-Pro-Pro-Gln-Asp-Asn-	9 (2%)	23
-Arg-Arg-Ala-Pro-Gln-Asp-Arg-	8 (2%)	24

Together these sequences account for 85% of Epitope III variants, with the balance comprised of a large number of low incidence variations.

Epitope III sequences were only available from 18 examples of HIV-1 non-B subtypes. Unlike Epitope I, they showed divergence from the B subtype sequences and optionally a larger number of sequences can be selected for inclusion into the composition of this invention, if additional non-B subtype Epitope III sequences are determined and are desirable in an immunogenic composition of this invention.

B. Assessment of Immunological Reactivity and Cross Reactivity of Selected Epitope III Sequences

Immunizing and detector sequences were synthesized, as described in Example 1, for the sequences in Table 18. Rabbits were immunized and antiserums were tested by ELISA, as described in Example 4, for reactivity and cross-reactivity. The various antiserums and detector peptides were utilized to determine immunogenicity of the various sequences and the extent of immunological cross reactivity. The incidence and immunological reactivity of Epitope III sequences of the formula -Arg-Arg-X-Pro-Gln-Y-Ser- (SEQ ID NO: 10) (see above) are shown in Table 18. In Table 18 below, percent cross-reactivity was measured with antiserum to Cys-Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser- (SEQ ID NO: 74), self titer =46,115. The results of Table 18 demonstrate that immunization with -Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gin-Asp-Ser- (SEQ ID NO: 29) should provide effective cross-presented in 61% of HIV-1 strains.

TABLE 18

Epitope III Sequence	# per 482 sequences	% Cross-reactivity	SEQ ID NO.
-Arg-Arg-Ala-Pro-Gln-Asp-Ser-	93	100	19
-Arg-Arg-Pro-Pro-Gln-Asp-Ser-	50	111	75
-Arg-Arg-Pro-Pro-Gln-Asn-Ser-	41	96	76
-Arg-Arg-Pro-Pro-Gln-Gly-Ser-	37	97	77
-Arg-Arg-Ser-Pro-Gln-Asp-Ser-	19	93	73
-Arg-Arg-Thr-Pro-Gln-Gly-Ser-	14	56	68
-Arg-Arg-Ala-Pro-Gln-Gly-Ser-	9	87	69
-Arg-Arg-Thr-Pro-Gln-Asp-Ser-	7	116	70
-Arg-Arg-Ala-Pro-Gln-Asn-Ser-	5	128	72
-Arg-Arg-Ser-Pro-Gln-Asp-Ser-	4	110	73
-Arg-Arg-Ser-Pro-Gln-Asn-Ser-	2	142	78
-Arg-Arg-Ala-Pro-Gln-Ser-Ser-	1	97	79
-Arg-Arg-Ser-Pro-Gln-Gly-Ser-	1	78	80
-Arg-Arg-Thr-Pro-Gln-Asn-Ser-	1	43	81
	292/482 (61%)		

Immunization with -Arg-Arg-Ala-Pro-Pro-Asp-Asn- (SEQ ID NO: 50) and -Arg-Arg-Ala-Pro-Pro-Asp-Ser- (SEQ ID NO: 20) yielded antibodies that cross-reacted with both detector peptides, as shown in Table 19. Thus, inclusion of either sequence in an immunizing composition of this invention provides antibodies against Tat protein Epitope III variants in a further 41/482 (8.5%) of HIV-1 strains.

Immunization with -Arg-Gln-Arg-Arg-Arg-Ala-His-Gln-Asn-Ser- (SEQ ID NO: 52) (20/482 (4%)) induced antibodies that gave a self titer of 209,286 and a cross-reactivity of 5,356 (2.5%) with -Arg-Arg-Ala-His-Gln-Asp-Ser- (SEQ

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This was accepted since this change does not affect antibody binding of Epitope III (see Example 4).

This gene was then excised with restriction enzymes and inserted into the expression vector pBAD (L-M. Guzman et al., J. Bacteriol, 177:4121 (1950)) containing, in frame, the sequence for green fluorescent protein (GFP) (A. Crameri et al., Nature Biotech, 14:315 (1996)). TG1 *E. coli* were transfected and green-fluorescent colonies were isolated.

The isolated colonies were grown and expression was induced. Protein from each of three colonies had fluorescent bands on Western blotting with the expected molecular size (i.e., twice that of GFP alone). The resulting protein was soluble and was purified by nickel column affinity purification utilizing a hexa-histidyl that had been incorporated in the sequence.

Yield was approximately 1 mg protein per liter of supernatant after double affinity purification to yield >90% purity.

EXAMPLE 8

Immunological Characterization of the Recombinant Fusion Protein Expressing HIV-1 Tat Protein Epitope Variants

A. Reactivity of Fusion Protein with Rabbit Antiserums to Epitope I and 2 Variants.

Rabbit antiserums generated to synthetic peptides corresponding to the four primary Epitope I sequences and four Epitope III sequences (see below) were tested by ELISA, using the methodology described in Examples 1 and 4, except that the plates were initially directly coated with a 100 μg/ml solution of antiserums with the fusion protein.

TABLE 22

Antiserum to:	Titers on fusion protein	SEQ ID NOS
-Val-Asp-Pro-Arg-Leu-Glu-Pro-Trp-Lys-His-Pro Gly-Ser-	>>8000	28
-Val-Asp-Pro-Asn-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-	>>8000	47
-Val-Asp-Pro-Lys-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-	>>8000	45
-Val-Asp-Pro-Ser-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-	>8000	46
-Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser-	7000	29
-Arg-Gln-Arg-Arg-Arg-Ala-His-Gln-Asn-Ser-	>>8000	52
-Arg-Gln-Arg-Arg-Arg-Pro-Pro-Gln-Asp-Ser-	>8000	53
-Arg-Gln-Arg-Gln-Arg-Ala-Pro-Asp-Ser-Ser-	8000	82

These data show that the variant epitope sequences, expressed as a linear recombinant fusion protein, are expressed in a conformation recognizable by antibodies to the corresponding synthetic peptides.

B. Immunization of Mice with the Fusion Protein

Three mice were immunized with 10 μg each of an aqueous solution of the fusion protein of Example 7 emulsified with an equal volume of Freund's complete adjuvant, given intraperitoneally. Two weeks later they were similarly boosted, except that Freund's incomplete adjuvant was used. Serums were obtained three weeks later.

C. ELISA Testing of Antiserums to the Fusion Protein with Synthetic Peptides Corresponding to the Epitope Variants Incorporated in the Fusion Protein

ELISA testing was performed as described in Example 1 except that horseradish peroxidase conjugated anti-mouse immunoglobulin was used to detect antibody binding. The results are summarized in Table 23 below. These data demonstrate that both Epitope I and Epitope III sequences are expressed in the linear fusion protein, and react with antibodies to the synthetic sequences (see above). Antibodies to Epitope I were detectably induced by the recombinant fusion protein under the conditions of this experiment in mice. The failure of Epitope III sequences expressed within this linear peptide is in keeping with the findings with synthetic peptides, wherein C-terminal extension of the immunogen diminishes the resulting antibody titer (see Example 4).

TABLE 23

Detector peptides	Titer with antiserum to fusion protein	SEQ ID NO
-Val-Asp-Pro-Arg-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser	2218	28
-Val-Asp-Pro-Asn-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser	3158	47
-Val-Asp-Pro-Lys-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser	2440	45
-Val-Asp-Pro-Ser-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser	3031	46
-Val-Asn-Pro-Ser-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser	3718	48
-Val-Asp-His-Arg-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser	3223	49
-Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser-	background	29
-Arg-Gln-Arg-Arg-Arg-Ala-His-Gln-Asn-Ser-	"	52
-Arg-Gln-Arg-Arg-Arg-Pro-Pro-Gln-Asp-Ser-	"	53
-Arg-Gln-Arg-Gln-Arg-Ala-Pro-Asp-Ser-Ser-	"	82
-Arg-Gln-Arg-Arg-Arg-Ala-Pro-Glu-Asp-Ser-	"	83
-Arg-Gln-Arg-Arg-Arg-Pro-Pro-Gln-Gly-Ser-	"	59
-Arg-Gln-Arg-Arg-Gly-Pro-Pro-Gln-Gly-Ser-	"	60
-Arg-Gln-Arg-Arg-Arg-Pro-Pro-Gln-Asn-Ser-	"	61
-Arg-Gln-Arg-Arg-Arg-Ser-Pro-Gln-Asp-Ser-	"	62
-Arg-Gln-Arg-Arg-Arg-Ser-Pro-Gln-Asn-Ser-	"	63
-Arg-Gln-Arg-Arg-Arg-Thr-Pro-Gln-Ser-Ser-	"	64
-Arg-Gln-Arg-Arg-Arg-Ala-His-Gln-Asp-Ser-	"	65
-Arg-Gln-Arg-Arg-Arg-Ala-Pro-Pro-Asp-Ser-	"	66

EXAMPLE 9

Primate Animal Study

A study was conducted in ten juvenile male rhesus macaques to determine if the presence of antibodies to Tat protein, induced by a synthetic peptide of this invention prior to infection with immunodeficiency virus, would attenuate infection and reduce levels of virus in plasma. HIV-1 does not infect monkeys, but a corresponding simian immunodeficiency virus (SIV) does. P. A. Luciw et al., Proc. Natl. Acad. Sci. USA, 92:7490 (1995) constructed an infectious recombinant virus (chimera) of SIV_{mac239} and HIV-1_{SF33} that does infect monkeys, typically causing an acute viremia that peaks around 2 weeks and subsequently subsides by week 8. In this chimeric construct, termed $SHIV_{SF33}$, the SIV nucleotides encoding tat, rev and env (gp160) of SIV_{mac239} have been replaced with the corresponding region of HIV-1_{SF33}.

A. Immunization of Monkeys

The monkeys were randomized into two groups.

Each monkey of group 1 (control group) was immunized with 0.4 mg diphtheria toxoid (Commonwealth Serum Laboratories, Victoria, Australia) with 0.25 mg threonyl muramyl dipeptide (T-MDP) in 0.5 ml water, this being emulsified with 0.5 ml ME75 adjuvant (Chiron Corp, Emeryville Calif.).

Each monkey of group 2 (test group) was immunized with 0.1 mg of the synthetic peptide Cys-Val-Asp-Pro-Asn-Leu-Glu-Pro-Trp-His-Pro-Gly-Ser-amide (SEQ ID NO: 84)

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TABLE 27

	Week 4	Week 8	Week 12	Week 16
GML Viral load- controls	17,000	900	400	120
(# negative)	(0/5)	(0/5)	(0/5)	(1/5)
GML Viral load - tests	5,000	300	8	4
(# negative)	(0/9)	(0/9)	(6/9)	(7/9)
% inhibition	71%	67%	98%	97%

Seroconversion to SIV positive occured at 4–8 weeks. Thus the post seroconversion viral loads (the most significant prognostic marker in HIV-1 infection) were significantly lowered in the presence of antibodies to HIV-1 Tat protein.

In an effort to understand the high peak viral load at 2 weeks in the test group, scrums were tested at the time of viral challenge (2 weeks post the last immunization) for TNFα by ELISA. TNFα, which is released during an immune response, activates cells and is known to by-pass the requirement for Tat mediated activation to support HIV-1 proliferation. The inventor determined that whereas serum TNFα was undetectable pre-immunization, it was detected in all Tat immunized monkeys 2 weeks post immunization with a mean level of 7 pg/ml.

It was concluded that the effects of Tat interdiction on the acute infection were masked by the peri-immunization TNFα activation; once this subsided, the Tat immunized group developed significantly lower viral loads, with the majority having undetectable (<100 copies/ml) levels in plasma.

EXAMPLE 11

Method and Kits for Detecting Titers and Specificities of Antibodies Induced by Vaccination

To follow the titer and specificities of antibodies induced following immunization with the vaccines of this invention, an assay method may be employed. In one embodiment of such as assay, peptides containing the sequences reported in Table 28 (depending on the composition of the immunizing vaccine) are used to develop kits measuring titers and reactivity patterns of antibodies in vaccinated subjects.

TABLE 28

Epitope	Sequence	SEQ ID NOS
I	-Glu-Pro-Val-Asp-Pro-Arg-Leu-Glu-Pro-	(amino acids 2–10 of SEQ ID NO: 1)
I	-Glu-Pro-Val-Asp-Pro-Lys-Leu-Glu-Pro-	118
I	-Glu-Pro-Val-Asp-Pro-Ser-Leu-Glu-Pro-	119
I	-Glu-Pro-Val-Asp-Pro-Asn-Leu-Glu-Pro-	120
II	-Lys-Gly-Leu-Gly-Ile-Ser-Tyr-Gly-Arg-Lys-Lys-	(amino acids 41–51 of SEQ ID NO: 1)
III	-Arg-Arg-Ala-Pro-Pro-Asp-Ser-	(amino acids 266–272 of SEQ ID NO: 3)
III	-Arg-Arg-Ala-His-Gln-Asp-Ser-	121
III	-Arg-Arg-Ala-Pro-Gln-Asp-Ser-	19
IV	-Ser-Gln-Thr-His-Gln-Val-Ser-Leu-Ser-Lys-Gln-Pro-	122

These peptides are synthesized with Biotin-Ser-Gly-Ser-Gly- (SEQ ID NO: 123) at the N-terminus. Each peptide is coated onto separate avidin coated plates, with a sequence -Ser-Gly-Ser-Gly- (SEQ ID NO: 30) serving as a spacer to ensure that the relevant peptide sequence is external to the biotin binding pocket of avidin. The plates are then incubated with dilutions of test serum, washed, and the antibody binding determined with reagent to human immunoglobulin, e.g., rabbit anti-human immunoglobulin, bound to, e.g., biotin, or directly labeled with enzyme. An avidin-enzyme complex is used to detect the biotin label, or a reagent employed to react with the enzyme and produce a calorimetric signal (R&D kit inserts).

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

```
(1) GENERAL INFORMATION:

   (iii) NUMBER OF SEQUENCES: 124

(2) INFORMATION FOR SEQ ID NO: 1:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 72 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: <Unknown>
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
1               5                   10                  15

Gln Pro Lys Thr Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe
            20                  25                  30

His Cys Gln Val Cys Phe Thr Thr Lys Gly Leu Gly Ile Ser Tyr Gly
```

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METHODS AND COMPOSITIONS FOR IMPAIRING MULTIPLICATION OF HIV-1

CROSS-REFERENCE TO OTHER INVENTIONS

This is a divisional of U.S. Patent Application No. 09/451,067, filed November 30, 1999, which is a divisional of U. S. Patent Application No. 09/113,921, filed July 10, 1998, now U. S. Patent No. 6,193,981, issued February 27, 2001, which is a continuation-in-part of United States patent application No. 08/893,853, filed July 11, 1997, now U. S. Patent No. 5,891,994, issued April 6, 1999.

BACKGROUND OF THE INVENTION

The present invention relates generally to compositions and methods useful for inhibiting the multiplication of human immunodeficiency virus-1 (HIV-1) in infected patients, symptomatic or asymptomatic, and for attenuating HIV-1 multiplication during primary infection in previously uninfected subjects, thus minimizing progression to AIDS.

High plasma levels of human immunodeficiency virus type 1 (HIV-1) RNA are found during primary infection with HIV-1, the seroconversion illness, (C. Baumberger *et al*, AIDS, 7:(suppl 2):S59 (1993); M. S. Saag *et al*, Nature Med., 2:625 (1996)), after which they subside as the immune response controls the infection to a variable extent. Post seroconversion, lower but detectable levels of plasma HIV-1 RNA are present, and these levels rise with disease progression to again attain high levels at the AIDS stage (M. S. Saag *et al*, Nature Med., 2:265 (1996)). Approximately 50% of subjects have a symptomatic illness at seroconversion (B. Tindall and D. A. Cooper, AIDS, 5:1 (1991)) and symptomatic seroconversion is associated with an increased risk for the development of AIDS, probably because a severe primary illness is likely related to an early and extensive spread of HIV.

Inhibition of viral multiplication during the initial infection will likely reduce the subsequent development of chronic viremia leading to AIDS. Current medical practice, with administration of antiviral drugs for defined "at risk" situations,

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such as needle sticks with contaminated blood or pregnancy in HIV infected mothers, supports this concept.

Post seroconversion levels of HIV-1 RNA in plasma have proven to be the most powerful prognosticator of the likelihood of progression to AIDS (J. W. Mellors *et al*, Science, 272:1167 (1996); M. S. Saag *et al*, Nature Med., 2:265 (1996); R. W. Coombs *et al*, J. Inf. Dis., 174:704 (1996); S. L. Welles *et al*, J. Inf. Dis., 174:696 (1990)). Other measures of viral load, such as cellular RNA (K. Saksela *et al*, Proc. Natl. Acad. Sci. USA, 91:1104 (1994)) and cellular HIV proviral DNA (T-H. Lee *et al*, J. Acq. Imm. Def. Syndromes, 7:381 (1994)) similarly establish the importance of the initial infection in establishing viral loads that determine future disease progression.

Thus, any intervention that inhibits HIV-1 infectivity during initial infection and/or lowers viral load post sero-conversion is likely to have a favorable influence on the eventual outcome, delaying or preventing progression to AIDS.

A variety of methods are now employed to treat patients infected with human immunodeficiency virus (HIV-1), including treatment with certain combinations of protease inhibitor drugs. Unfortunately, however, this type of treatment is associated with serious side effects in some patients. Alternatively, vaccines are under development for control of the spread of HIV-1 to uninfected humans. However, this effort has largely been directed to proteins of the virus, expressed on the surface of infected cells, which are recognized by cytotoxic T cells with elimination of the infected cells, while free virus is blocked and cleared by antibody to surface antigens of the virion. Limitations of this mode of vaccination are readily apparent for HIV-1, which has demonstrated a great diversity in immunogenic viral epitopes and rapid mutational variations that occur within and between individuals (B. D. Preston et al., *Science*, 242:1168(1988); J. D. Roberts et al., *Science*, 242:1171 (1988); A. R. Meyerhans et al., *Cell*, 58:901 (1989); K. Kusumi et al., *J. Virol.*, 66:875 (1992); B. A. Larder et al., *Science*, 243:1731 (1989); M. S. Sang et al., *N. Engl. J. Med.*, 329:1065 (1993); M. A. Sande, et al., *JAMA*, 270:2583 (1993); M. Seligmann et al., *Lancet*, 343:871 (1994); G. Meyers et al., *Human retroviruses and AIDS 1993, I-V.*

specification demonstrates that a number of peptides corresponding to these Tat sequences and the corresponding integrins block *in vitro* cell binding to Tat coated plates, as do antibodies to the appropriate integrins. However, the specification also shows that these reagents do not block uptake of functional Tat by cells (see Example 9 in WO92/14755), thus nullifying the proposed mechanism of action for therapeutic benefit in HIV infection. The Tat sequences described in this international application are distinct from the peptide immunogens of the present invention.

Both monoclonal and polyclonal antibodies to Tat protein have been readily produced in animals and shown to block uptake of Tat protein *in vitro* (see, e.g., D. Brake et al, J. Virol., 64:962 (1990); D. Mann et al, EMBO J., 10:1733 (1991); J. Abraham et al, cited above; P. Auron et al, cited above; M. Jaye et al, cited above; G. Zauli et al, cited above). More recent reports showed that monoclonal or polyclonal antibodies to Tat protein added to tissue culture medium attenuated HIV-1 infection *in vitro* (L. Steinaa et al, Arch. Virol., 139:263 (1994); M. Re et al, J. Acq. Imm. Def. Syndr. Hum. Retrovirol., 10:408 (1995); and G. Zauli et al, J. Acq. Imm. Def. Syndr. Hum. Retrovirol., 10:306 (1995)).

The inventor's own publication (G. Goldstein, Nature Med., 2:960 (1996); see also, International Patent Publication No. WO95/31999, published November 30, 1995) reviewed the evidence indicating that secretion of HIV-1 Tat protein from infected cells and uptake by both infected and uninfected cells was important for the infectivity of HIV-1. Previous studies also showed that antibodies to Tat protein *in vitro* blocked uptake of Tat and inhibited *in vitro* infectivity. Goldstein proposed active immunization of mammals to induce antibodies to HIV-1 Tat protein as a potential AIDS vaccine.

Despite the growing knowledge about HIV-1 disease progression, there remains a need in the art for the development of compositions and methods for treatment of HIV-1, both prophylactically and therapeutically, which are useful to lower the viral levels of HIV-1 for the treatment and possible prevention of the subsequent, generally fatal, AIDS disease.

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SUMMARY OF THE INVENTION

In one aspect, the invention provides as a novel composition comprising a peptide or polypeptide, which comprises an amino acid sequence selected from the formula referred to as Epitope I: R1-Val-Asp-Pro-Y-Leu-Glu-Pro-R2 (SEQ ID NO: 36), wherein Y is variously Arg, Lys, Ser or Asn. The N-terminal R1 may represent hydrogen (i.e., the hydrogen on the unmodified N terminal amino acid), or a lower alkyl, or a lower alkanoyl. R1 may also include a sequence of between 1 to about 5 amino acids, optionally substituted with a lower alkyl or lower alkanoyl. In one embodiment, R1 is -X-Pro-, wherein X is Glu or Asp. Preferably, R1 represents 2 amino acids. The C-terminal R2 can also represent the hydroxyl group on the C terminal amino acid or an amide. To enhance titer R2 is preferably a sequence of between 1 to about 14 additional amino acids amidated at the carboxyl terminus. In a preferred embodiment, R2 is -Trp-Lys-His-Pro-Gly-Ser- amide (SEQ ID NO: 10). The peptides or polypeptides of these compositions are produced synthetically or recombinantly. This composition may take the form of one or more of the above-described peptides expressed as a synthetic peptide coupled to a carrier, or expressed as a multiple antigenic peptide, or the selected peptides may be expressed within a recombinantly produced protein. This composition is designed to induce antibodies reactive with greater than 95% of the known variants of the HIV-1 Tat protein.

In another aspect, the above-described composition further contains one or more additional peptide or polypeptide(s) which represent other amino acid sequences which correspond to amino acid residues 2 or 4 to 10 of an HIV-1 Tat protein. These optional amino acid sequences are described in detail below. These sequences are preferably from an HIV-1 strain with a Tat protein variant at that location.

In another aspect, the invention provides a novel composition comprising a peptide or polypeptide of the formula referred to as Epitope II: R3-Lys-X-Leu-Gly-Ile-Ser-Tyr-Gly Arg-Lys-Lys- R4 (SEQ ID NO: 37). According to this formula, X is Gly or Ala. The N terminal R3 may represent hydrogen (i.e., the hydrogen on the unmodified N terminal amino acid), or may be a lower alkyl, or a

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lower alkanoyl. R3 may also include a sequence of between 1 to about 5 amino acids, optionally substituted with a lower alkyl or lower alkanoyl. The C terminal R4 may be the free hydroxyl of the C terminal amino acid, or an amide, or a sequence of one or up to about 5 additional amino acids, optionally substituted with an amide. The peptides or polypeptides of these compositions are produced synthetically or recombinantly, provided that the recombinant Epitope II peptide is situated at the C terminus of the recombinant protein. This composition may take the form of one or more of the above-described peptides expressed as a synthetic peptide coupled to a carrier, or expressed as a multiple antigenic peptide. This composition is designed to induce antibodies reactive with greater than about 95% of the known variants of HIV-1 Tat protein.

In yet a further aspect, this invention provides a composition comprising a peptide or polypeptide of the formula referred to as Epitope III: R5-Arg-Arg-X-Z-A-Y-Ser-R6 (SEQ ID NO: 38), wherein X is selected from the group consisting of Ala, Pro, Ser and Gln; wherein Y is selected from the group consisting of Asp, Asn, Gly and Ser; wherein Z is selected from the group consisting of Pro and His; and wherein A is selected from the group consisting of Gln and Pro. The N terminal R5 is hydrogen, a lower alkyl, a lower alkanoyl, or a sequence of between 1 to about 3 amino acids, optionally substituted with a lower alkyl or lower alkanoyl. In a preferred embodiment R5 is -Gln-Arg-, optionally modified as above. The C terminal R6 is either a free hydroxyl or an amide. A preferred embodiment of such a composition contains at least three Epitope III peptides, i.e.,-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser- (amino acids 54-62 of SEQ ID NO: 1), -Gln-Arg-Arg-Arg-Ala-His-Gln-Asp-Ser- (amino acids 2-10 of SEQ ID NO: 65), and -Gln-Arg-Arg-Arg-Ala-Pro-Pro-Asp-Ser- (amino acids 264-272 of SEQ ID NO: 3), optionally modified as above. Other peptides or polypeptides representative of amino acids 56-62 of Tat, but having different sequences from that of the above formula may also be included in the composition. The peptides or polypeptides of these compositions are produced synthetically or recombinantly. This composition may take the form of one or more of the above-described peptides expressed as a synthetic peptide coupled to a carrier, or

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expressed as a multiple antigenic peptide, or the selected peptides may be expressed within a recombinantly produced protein. This composition is designed to induce antibodies reactive with greater than about 75% of all known variants of HIV-1 Tat protein.

In still a further aspect, this invention provides a composition comprising a peptide or polypeptide of the formula referred to as Epitope IV: R7-Ser-Gln-X-His-Gln-Y-Ser-Leu-Ser-Lys-Gln-Pro-R8 (SEQ ID NO: 39), wherein X is selected from the group consisting of Asn and Thr; and wherein Y is selected from the group consisting of Ala and Val. The N terminal R7 may be hydrogen, a lower alkyl, a lower alkanoyl, or a sequence of between 1 to about 3 amino acids, optionally substituted with a lower alkyl or lower alkanoyl. The C terminal R8 may be a free hydroxyl, an amide, or a sequence of one or up to about 3 additional amino acids, optionally substituted with an amide. A preferred Epitope IV peptide is -Ser-Gln-Thr-His-Gln-Ala-Ser-Leu-Ser-Lys-Gln-Pro- (SEQ ID NO: 40). The peptides or polypeptides of these compositions are produced synthetically or recombinantly. This composition may take the form of one or more of the above-described peptides expressed as a synthetic peptide coupled to a carrier, or expressed as a multiple antigenic peptide, or the selected peptides may be expressed within a recombinantly produced protein. This composition is designed to induce antibodies reactive with greater than 64% of all known variants of HIV-1 Tat protein.

In still another aspect, this invention provides composition described above that contains peptides or polypeptides which comprise one or more Epitope I peptides in combination with one or more Epitope II peptides, and/or one or more Epitope III peptides, and/or one or more Epitope IV peptides. Such compositions can combine appropriate Epitope peptides, so as to provide for a composition than induces antibodies reactive with greater than about 99% of all known HIV-1 Tat proteins.

In yet a further aspect, the invention provides a synthetic gene which encodes sequentially a peptide or polypeptide that contains at least one Epitope I amino acid sequence defined above, optionally with a carboxy terminal Epitope II peptide, or contains at least two Epitope I amino acid sequences. The synthetic gene

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Table 1

Immunizing Peptides	Detector Peptides (GMT (% self-binding))			
Position Y	Arg	Lys	Ser	Asn
Arg	77,000 (100)	10,000 (13)	10,000 (13)	9,000 (12)
Lys*	51,000 (62)	82,000 (100)	35,000 (43)	45,000 (55)
Ser	8,000 (6)	8,000 (6)	128,000 (100)	14,000 (11)
Asn*	17,000 (13)	12,000 (9)	61,000 (46)	134,000 (100)

* Only one high titer antiserum available.

Preferably a composition of this invention contains one or more of the following Epitope I peptides or polypeptides:

R1-Val-Asp-Pro-Arg-Leu-Glu-Pro-R2 (SEQ ID NO: 6);

R1-Val-Asp-Pro-Lys-Leu-Glu-Pro-R2 (SEQ ID NO: 7);

R1-Val-Asp-Pro-Ser-Leu-Glu-Pro-R2 (SEQ ID NO: 8);

R1-Val-Asp-Pro-Asn-Leu-Glu-Pro-R2 (SEQ ID NO: 9).

As demonstrated above, the immunogen in which Y is Lys (SEQ ID NO: 7) induces antibodies with good reactivity with the three other variants. No immunogen induced high titer antibodies with good cross-reactivity with the variant in which Y was Ser. Thus an immunogen of Epitope I in which Y was Lys (SEQ ID NO: 7) may suffice for full cross-reactivity to all four position Y variants, and may be used alone in an immunogenic composition. While this pattern of response of the peptide in which Y is Lys occurs in the majority of tests to date, it should be expected by one of skill in the art, that some differences in cross-reactivity from the results above may occur in some test samples.

Alternatively, compositions of this invention comprise two, three or all four of these amino acid sequences (SEQ ID NOS: 6-9). Alternatively, a combination of Epitope I immunogens in which Y was Lys and in which Y was Asn

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to AIDS. Active induction of antibodies in the early asymptomatic phase of HIV infection may reduce viral multiplication, lower the plasma viral load and reduce the likelihood of progression to AIDS. The composition which contains at least one Epitope I immunogen up to all four of the SEQ ID NO: 6-9 amino acid sequences, can elicit an immune response to about 97% of the 400 known Tat sequences of the common B subtypes of HIV-1 and with Tat proteins of all 18 non-B subtype HIV-1 that have been sequenced (courtesy of Dr. Esther Guzman, Los Alamos NIAID HIV database; GenBank database).

B. *Epitope II Immunogenic Compositions*

In another embodiment, the present invention provides a composition comprising at least one Epitope II amino acid sequence. This Epitope II sequence elicits a specific humoral immune response (for the purpose of this invention) in a mammal exposed to the Epitope II sequence *in vivo*. Epitope II defines peptides of the formula R3-Lys-X-Leu-Gly-Ile-Ser-Tyr-Gly-Arg-Lys-Lys-R4, wherein X is Gly (70%) or Ala (30%). This sequence is highly conserved. The immunogen in which X is Gly induces antibodies cross-reactive with the sequence in which X is Ala.

The N terminal R3 may represent the hydrogen on the unmodified N terminal amino acid Lys, or R3 may be a lower alkyl, or a lower alkanoyl, such as an acetyl group, substituent on the Lys. R3 may also include a sequence of between 1 to about 5 amino acids, optionally substituted with a lower alkyl or lower alkanoyl. The C terminal R4 may represent the free hydroxyl of the C terminal amino acid Lys, or R4 may be an amide on that C terminal amino acid. R4 may optionally be a sequence of one or up to about 5 additional amino acids, optionally substituted with an amide. The presently preferred immunogen for Epitope II is -Lys-Gly-Leu-Gly-Ile-Ser-Tyr-Gly-Arg-Lys-Lys- (amino acids 41-51 of SEQ ID NO: 1). This would react/cross-react with greater than 96% of known HIV-1 Tat proteins.

Epitope II is poorly immunogenic when presented within other sequences. Thus, for optimal immunogenicity, this sequence is prepared as a synthetic peptide fused to, or coupled to, a carrier protein or as a multiple antigenic peptide,

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optionally coupled to carrier protein. Alternatively, Epitope II may be expressed as the C terminal sequence of a recombinant protein, which is optionally fused in frame to a carrier protein at its amino terminal sequence. In a composition of this invention, an Epitope II peptide is preferably presented alone or in combination with one or more Epitope I peptides. Other compositions may employ one or more Epitope III or IV peptides.

C. Epitope III Immunogenic Compositions

In another embodiment, the present invention provides a composition comprising at least one, and preferably two or more Epitope III amino acid sequences. These Epitope III sequences elicit a specific humoral immune response (for the purpose of this invention) in a mammal exposed to the Epitope III sequences *in vivo*. This epitope shows considerable more variation than Epitopes I and II. These Epitope III immunogenic peptides and polypeptides are derived from Tat variant protein sequences corresponding to amino acids 56-62 of SEQ ID NO: 1. Epitope III defines peptides of the formula: R5-Arg-Arg-X-Z-A-Y-Ser-R6 (SEQ ID NO: 38), wherein X may be Ala, Pro, Ser or Gln; Y may be Asp, Asn, Gly or Ser; Z may be Pro or His; and A may be Gln or Pro. The Epitope III immunogens in which X is Ala induce antibodies that cross-react with the other position X variants. Epitope III immunogens containing Asp in position Y induce antibodies that cross-react with the other position Y variants. The three most common variants for positions Z and A are -Pro-Gln- (61%), -Pro-Pro- (8%) and -His-Gln- (8%). Antibodies induced by these three immunogens do not cross-react with the others so that three immunogens would need to be used to cover these variants (77%).

According to the formula of Epitope III above, the seven amino residues which form the minimum reactive Epitope III sequences, may be flanked by other amino acids, so that the entire Epitope III sequence may be between 7 and about 15 amino acids in length. As indicated in Example 3 below, the identity of the flanking amino acids is not essential to the biological function of the Epitope III immunogen. In particular additional amino acids on the N-terminus of Epitope III sequences do not affect immunogenicity. The N terminal R5 may optionally represent the hydrogen on

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more different Epitope III immunogens, optionally with at least one Epitope I immunogen, and optionally with one or more Epitope II or Epitope IV immunogens.

D. Epitope IV Immunogenic Compositions

In another embodiment, the present invention provides a composition comprising at least one, and preferably two or more Epitope IV amino acid sequences. These Epitope IV sequences elicit a specific humoral immune response (for the purpose of this invention) in a mammal exposed to the Epitope IV sequences *in vivo*. The Epitope IV immunogenic peptides and polypeptides are derived from Tat variant protein sequences corresponding to amino acids 62-72 of SEQ ID NO: 1, including a C-terminal Pro from Exon 2 of HIV-1 Tat. Epitope IV defines peptides of the formula: R7-Ser-Gln-X-His-Gln-Y-Ser-Leu-Ser-Lys-Gln-Pro-R8 (SEQ ID NO: 39), wherein X may be Asn or Thr; and Y may be Ala or Val. The immunogen in which X is Thr induces antibodies that cross-react with the immunogen in which X is Asn. The immunogen in which Y is Val induce antibodies that do not cross-react with the peptides in which Y is Ala. However, the peptides containing Ala in position Y induce antibodies that cross-react with peptides for Epitope IV in which Y is Val. Thus the optimal Epitope IV immunogen is Ser-Gln-Thr-His-Gln-Ala-Ser-Leu-Ser Lys-Gln-Pro (SEQ ID NO: 40) and this induces antibodies reactive/cross-reactive with 64% of known HIV-1 Tat proteins.

According to the formula of Epitope IV above, the twelve amino residues which form the minimum reactive Epitope IV sequences, may be flanked by a few other amino acids, so that the entire Epitope IV sequence may be between 12 and about 18 amino acids in length. The N terminal R7 may represent the hydrogen of the N terminal amino acid, or a lower alkyl or alkanoyl, such as an acetyl group, substituent on the N terminal amino acid. Although N-terminal extension markedly inhibits immunogenicity, the R7 may also be a sequence of between 1 to about 3 amino acids, optionally substituted with a lower alkyl or lower alkanoyl. The C terminal R8 may represent the free hydroxyl on the C terminal amino acid, or an amide substituent on the C terminal amino acid, or R8 may be a sequence of one or up to about 3 additional amino acids, optionally substituted with an amide. Additionally,

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immunogen(s) (95%) with an Epitope II immunogen (96%) would result in antibodies in immunologically responsive subjects reactive with 99.8% of known HIV-1 Tat proteins. Thus, as one example, a composition of this invention contains one Epitope I (underlined)-Epitope II (double-underlined) fused peptide immunogen such as Cys-Glu-Pro-Val-Asp-Pro-Lys-Leu-Glu-Pro-Trp-Lys-Glu-Leu-Gly-Ile-Ser-Tyr-Gly-Arg-Lys-Lys-amide (SEQ ID NO: 67), coupled to carrier protein attached to Epitope I, or the same peptide (less the N-terminal Cys for coupling), synthesized as a multiple antigenic peptide, optionally coupled to a carrier protein. Alternatively, mixtures of two or more immunogens could be used as follows.

The Epitope I immunogens, with or without any Epitope II , III or IV or other optional immunogens, may be prepared and used in immunogenic compositions in a variety of forms, for example, chemically synthesized or as recombinant peptides, polypeptides, proteins, fusion proteins or fused peptides.

1. Synthetic Peptide/Protein Coupled to a Carrier

As one embodiment, a composition of the present invention may be a synthetic peptide, containing single or multiple copies of the same or different Epitope I immunogen amino acid sequences and/or Epitope II/III/IV immunogenic amino acid sequences, and optionally amino acid sequences of the optional immunogens, coupled to a selected carrier protein. In this embodiment of a composition of this invention, multiple above-described Epitope I amino acid sequences with or without flanking sequences, may be combined sequentially in a polypeptide and coupled to the same carrier. Alternatively, the Epitope I, II, III, or IV immunogens, may be coupled individually as peptides to the same or different carrier proteins, and the resulting immunogen-carrier constructs mixed together to form a single composition.

For this embodiment, the carrier protein is desirably a protein or other molecule which can enhance the immunogenicity of the selected immunogen. Such a carrier may be a larger molecule which has an adjuvanting effect. Exemplary conventional protein carriers include, without limitation, *E. coli* DnaK protein, galactokinase (galK, which catalyzes the first step of galactose metabolism in

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Epitope II or III peptide is fused to the C terminus of the Epitope I sequence and not further modified on its own C terminus. The synthetic gene may encode multiple copies of the same amino acid sequence, copies of multiple different immunogens or amino acid sequences, or multiple copies of multiple different immunogens or amino acid sequences. The synthetic gene may encode the selected amino acid sequences in an open reading frame with, or fused to, a nucleic acid sequence encoding a carrier protein. A further characteristic of the synthetic gene may be that it encodes a spacer between each sequence encoding an immunogen and/or between the sequence encoding an immunogen and the sequence encoding the carrier protein.

The synthetic gene of the present invention may also be part of a synthetic or recombinant molecule. The synthetic molecule may be a nucleic acid construct, such as a vector or plasmid which contains the synthetic gene encoding the protein, peptide, polypeptide, fusion protein or fusion peptide under the operative control of nucleic acid sequences encoding regulatory elements such as promoters, termination signals, and the like. Such synthetic molecules may be used to produce the polypeptide/peptide immunogen compositions recombinantly.

The synthetic gene or synthetic molecules can be prepared by the use of chemical synthesis methods or preferably, by recombinant techniques. For example, the synthetic gene or molecules may contain certain preference codons for the species of the indicated host cell.

The synthetic gene or molecules, preferably in the form of DNA, may be used in a variety of ways. For example, these synthetic nucleic acid sequences may be employed to express the peptide/polypeptides of the invention *in vitro* in a host cell culture. The expressed immunogens, after suitable purification, may then be incorporated into a pharmaceutical reagent or vaccine.

Alternatively, the synthetic gene or synthetic molecule of this invention may be administered directly into a mammalian, preferably human subject, as so-called 'naked DNA' to express the protein/peptide immunogen *in vivo* in a patient. See, e.g., J. Cohen, Science, 259:1691-1692 (March 19, 1993); E. Fynan et al., Proc. Natl. Acad. Sci., USA, 90:11478-11482 (Dec. 1993);and J. A. Wolff et al.,

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I, II, III, and/or IV immunogens and optional immunogens, as described above. Suitable mammals include primates, such as monkeys; smaller laboratory animals, such as rabbits and mice, as well as larger animals, such as horse, sheep, and cows. Such antibodies may also be produced in transgenic animals. However, a desirable host for raising polyclonal antibodies to a composition of this invention includes humans.

The polyclonal antibodies raised in the mammal exposed to the composition are isolated and purified from the plasma or serum of the immunized mammal by conventional techniques. Conventional harvesting techniques can include plasmapheresis, among others.

Such polyclonal antibody compositions may themselves be employed as pharmaceutical compositions of this invention. Alternatively, other forms of antibodies may be developed using conventional techniques, including monoclonal antibodies, chimeric antibodies, humanized antibodies and fully human antibodies. See, e.g., Harlow *et al.*, Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988); Queen *et al.*, Proc. Nat'l. Acad. Sci. USA, 86:10029-10032 (1989); Hodgson *et al.*, Bio/Technology, 9:421 (1991); International PCT Application PCT/GB91/01554, Publication No. WO92/04381 and International PCT Application PCT/GB93/00725, Publication No. WO93/20210). Other anti-Tat antibodies may be developed by screening hybridomas or combinatorial libraries, or antibody phage displays (W. D. Huse *et al.*, Science, 246:1275-1281 (1988)) using the polyclonal or monoclonal antibodies produced according to this invention and the amino acid sequences of the Epitope I, II, III, IV or optional immunogens.

These antibody compositions bind to greater than 95%, and preferably greater than 99% of known Tat protein variants of HIV-1, and prevent the Tat proteins from supporting further HIV-1 multiplication. Thus, these antibodies are useful in pharmaceutical methods and formulations described below.

J. Pharmaceutical Compositions of the Invention

As another aspect of this invention, a pharmaceutical composition useful for inducing antibodies that react with greater than 95%, preferably greater than 99%, of known HIV-1 Tat proteins and impair the multiplication of HIV-

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treatment of virus infections. These proteins may be combined in a single pharmaceutical preparation for administration. Suitable pharmaceutically acceptable carriers for use in an immunogenic proteinaceous composition of the invention are well known to those of skill in the art. Such carriers include, for example, saline, buffered saline, a selected adjuvant, such as aqueous suspensions of aluminum and magnesium hydroxides, liposomes, oil in water emulsions and others. Suitable adjuvants may also be employed in the protein-containing compositions of this invention. The present invention is not limited by the selection of the carrier or adjuvant.

Suitable vehicles for direct DNA, plasmid nucleic acid, or recombinant vector administration include, without limitation, saline, or sucrose, protamine, polybrene, polylysine, polycations, proteins, $CaPO_4$ or spermidine. See e.g, International Patent Publication No. WO94/01139 and the references cited above.

The peptide/polypeptide compositions and synthetic genes or molecules *in vivo* are capable of eliciting in an immunized host mammal, e.g., a human, an immune response capable of interdicting greater than about 95 to about 99 %of known extracellular Tat protein variants from HIV-1 and thereby lowering the viral levels.

Yet another pharmaceutical composition useful for impairing the multiplication of HIV-1 comprises an antibody composition as described in detail above. In a pharmaceutical composition, the antibodies may be carried in a saline solution or other suitable carrier. The antibody compositions are capable of providing an immediate, exogenously provided interdiction of Tat.

The preparation of these pharmaceutically acceptable compositions, from the above-described components, having appropriate pH isotonicity, stability and other conventional characteristics is within the skill of the art.

K. *Method of the Invention - Impairing Multiplication of HIV-1*

According to the present invention, a method for reducing the viral levels of HIV-1 involves exposing a human to the Tat antibody-inducing pharmaceutical compositions described above, actively inducing antibodies that react with greater than 95%, preferably greater than 99%, of known HIV-1 Tat proteins,

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B. Assessment of immunological reactivity and cross-reactivity of the four primary immunogens

Immunizing and detector sequences were synthesized, as described in Example 1, for the following sequences (SEQ ID NOS: 28 and 45 through 47, respectively):

-Val-Asp-Pro-Arg-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-,

-Val-Asp-Pro-Lys-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-,

-Val-Asp-Pro-Ser-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-,

-Val-Asp-Pro-Asn-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-.

Rabbits were immunized and the antiserums were tested by ELISA, as described in Example 1, for reactivity and cross-reactivity. Self-reactivities are summarized in Table 8.

Table 8

Immunogen and detector sequence	GMT	SEQ ID NO
-Val-Asp-Pro-Arg-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-	88,000	28
-Val-Asp-Pro-Lys-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-	132,000	45
-Val-Asp-Pro-Ser-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-	166,355	46
-Val-Asp-Pro-Asn-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser-	173,097	47

Cross-reactivities between these primary immunogens with varying amino acid residues at position 3 of Epitope I are displayed in Table 9. Note that the results reported below are averages with one poorly reactive antiserum.

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B. Assessment of immunological reactivity and cross reactivity of selected Epitope III sequences

Immunizing and detector sequences were synthesized, as described in Example 1, for the sequences in Table 18. Rabbits were immunized and antiserums were tested by ELISA, as described in Example 4, for reactivity and cross-reactivity. The various antiserums and detector peptides were utilized to determine immunogenicity of the various sequences and the extent of immunological cross reactivity. The incidence and immunological reactivity of Epitope III sequences of the formula -Arg-Arg-X-Pro-Gln-Y-Ser- (SEQ ID NO: 10) (see above) are shown in Table 18. In Table 18 below, percent cross-reactivity was measured with antiserum to Cys-Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser- (SEQ ID NO: 74), self titer = 46,115. The results of Table 18 demonstrate that immunization with -Arg-Gln-Arg-Arg-Arg-Ala-Pro-Gln-Asp-Ser- (SEQ ID NO: 29) should provide effective cross-reactivity with most of these variants, represented in 61% of HIV-1 strains.

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EXAMPLE 9 - PRIMATE ANIMAL STUDY

A study was conducted in ten juvenile male rhesus macaques to determine if the presence of antibodies to Tat protein, induced by a synthetic peptide of this invention prior to infection with immunodeficiency virus, would attenuate infection and reduce levels of virus in plasma. HIV-1 does not infect monkeys, but a corresponding simian immunodeficiency virus (SIV) does. P.A. Luciw et al., Proc. Natl. Acad. Sci. USA, 92:7490 (1995) constructed an infectious recombinant virus (chimera) of SIV_{mac239} and HIV-1_{SF33} that does infect monkeys, typically causing an acute viremia that peaks around 2 weeks and subsequently subsides by week 8. In this chimeric construct, termed $SHIV_{SF33,}$ the SIV nucleotides encoding tat, rev and env (gp160) of SIV_{mac239} have been replaced with the corresponding region of HIV-$1_{SF33.}$

A. Immunization of monkeys

The monkeys were randomized into two groups.

Each monkey of group 1 (control group) was immunized with 0.4 mg diphtheria toxoid (Commonwealth Scrum Laboratories, Victoria, Australia) with 0.25 mg threonyl muramyl dipeptide (T-MDP) in 0.5 ml water, this being emulsified with 0.5 ml MF75 adjuvant (Chiron Corp, Emeryville CA).

Each monkey of group 2 (test group) was immunized with 0.1 mg of the synthetic peptide Cys-Val-Asp-Pro-Asn-Leu-Glu-Pro-Trp-His-Pro-Gly-Ser-amide (SEQ ID NO: 84) coupled to 0.4 mg diphtheria toxoid (A. C. Lee *et al.*, Mol. Immunol., 17:749 (1980)). The conjugate was dissolved in 0.5 ml water containing 0.25 mg T-MDP and emulsified with 0.5 ml MF75 adjuvant.

Each monkey was immunized at day 0 and day 28 (week 4) with two 0.5 ml intramuscular injections at two distinct sites. The synthetic peptide immunogen contained the B cell Epitope I, Val-Asp-Pro-Asn-Leu-Glu-Pro-Trp-Lys-His-Pro-Gly-Ser- (SEQ ID NO: 115) of the Tat protein of SF33 HIV-1 that is incorporated in the $SHIV_{SF33}$ molecular clone that was used to challenge the monkeys (see above).

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human immunoglobulin, bound to, e.g., biotin, or directly labeled with enzyme. An avidin-enzyme complex is used to detect the biotin label, or a reagent employed to react with the enzyme and produce a colorimetric signal (R&D kit inserts).

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.